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# Papillary Tubal Hyperplasia. The Putative Precursor of Ovarian Atypical Proliferative (Borderline) Serous Tumors, Noninvasive Implants and Endosalpingiosis

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## Abstract

In contrast to the controversy regarding the terminology and behavior of ovarian noninvasive low-grade serous tumors (atypical proliferative serous tumor [APST] and serous borderline tumor [SBT]), little attention has been directed to their origin. Similarly, until recently, proliferative lesions in the fallopian tube have not been extensively studied. The recent proposal that ovarian high-grade serous carcinomas are derived from intraepithelial carcinoma in the fallopian tube prompted us to evaluate the possible role of the fallopian tube in the genesis of low-grade serous tumors. We have identified a lesion, designated “papillary tubal hyperplasia (PTH)”, characterized by small rounded clusters of tubal epithelial cells and small papillae, with or without associated psammoma bodies, that are present within the tubal lumen and which are frequently associated with APSTs. Twenty-two cases in this study were selected from a population-based study in Denmark of approximately 1000 patients with low-grade ovarian serous tumors in whom implants were identified on the fallopian tube. Seven additional cases were seen recently in consultation at The Johns Hopkins Hospital (JHH). These 7 cases were not associated with an ovarian tumor. Papillary tubal hyperplasia was found in 20 (91%) of the 22 cases in the Danish study. Based on this association of PTH with APSTs with implants and the close morphologic resemblance of PTH, not only to the primary ovarian APSTs but also to the noninvasive epithelial implants and endosalpingiosis, we speculate that the small papillae and clusters of cells from the fallopian tubes implant on ovarian and peritoneal surfaces to produce these lesions. The 7 JHH cases of PTH that were not associated with an ovarian tumor support the view that PTH is the likely precursor lesion. We propose a model for the development of ovarian and extraovarian low-grade serous

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proliferations (APST, noninvasive epithelial implants and endosalpingiosis) that postulates that all of these lesions are derived from PTH, which appears to be induced by chronic inflammation. If this hypothesis is confirmed, then it can be concluded that low- and high-grade ovarian tumors develop from tubal epithelium and involve the ovary secondarily.

## Introduction

Considerable controversy has surrounded the terminology and behavior of noninvasive low-grade ovarian serous tumors [atypical proliferative serous tumors (APSTs) and serous borderline tumors (SBTs)] but little attention has been directed towards determining their origin. The most contentious issues concerning their terminology were resolved in 2003 by the Borderline Ovarian Tumor conference, under the auspices of the National Cancer Institute, which deemed that the terms APST and SBT are synonymous[17]. Accordingly, APST and SBT are used interchangeably in this report. The recent revelation that intraepithelial carcinoma in the fallopian tube is the likely precursor of most, if not all, ovarian high-grade serous carcinomas prompted us to reconsider the fallopian tube as a possible site of origin of low-grade serous tumors.

Previously, we reported that mucosal and luminal calcifications frequently surrounded by bland epithelium in the fallopian tube, so-called “salpingoliths”, were significantly more frequently associated with APSTs than all other ovarian neoplasms ($p<0.001$)[16]. In that study, salpingoliths were found in 24% of stage I and in 51% of stage II and III APSTs. We were unable to determine whether these lesions arose in the fallopian tube and implanted on the ovary and peritoneum, arose in the ovary and implanted in the tube or that the tubal and ovarian lesions developed independently.

During the course of a large population-based study of ovarian low-grade serous tumors from Denmark, aimed at evaluating their clinicopathologic and epidemiologic features as well as identifying biomarkers to assist in the distinction of noninvasive from invasive implants, we noted the frequent association and close morphologic resemblance of a particular type of tubal hyperplasia designated “papillary tubal hyperplasia (PTH)” with APSTs. Papillary tubal hyperplasia is characterized by small rounded clusters of tubal epithelial cells and small papillae, with or without associated psammoma bodies, floating within the tubal lumen. The present study describes the morphologic features of this lesion and its association with ovarian APSTs, peritoneal implants and endosalpingiosis.

## Materials and Methods

### Case Selection

Twenty-two cases from a population-based study of approximately 1000 ovarian low-grade serous tumors in Denmark and 7 recent cases of PTH seen in consultation at The Johns Hopkins Hospital (JHH) Department of Pathology, Division of Gynecologic Pathology constitute this descriptive analysis. Clinical information was obtained from a review of medical records and pathology reports from the Danish cases and from consultation letters, pathology reports and communication with the referring pathologists or clinicians for the JHH consultation cases. From 1 to 4 sections from the fallopian tubes were examined in the Danish study and 2–21 sections from the JHH consult cases. It should be noted that in all cases the sections were representative sections only.

**Danish cases—**The aims of the Danish study were to evaluate the clinicopathologic and epidemiologic features of ovarian low-grade serous tumors in the female population of Denmark. One specific aim was to determine if immunohistochemical and molecular genetic

analysis could distinguish noninvasive from invasive implants. This will be the subject of a future report. From among the 1000 tumors that qualified for the diagnosis of APST a subset of cases with implants in the mesosalpinx in which fallopian tubes were available for microscopic review were selected for further study. It should be noted that although one of us (RJK) was aware of these tubal proliferations and their association with APSTs for some time, it was not the intent at the start of the Danish study to evaluate them. It was only during the course of selecting cases for immunohistochemical and molecular genetic analysis of the implants that we were again struck by the resemblance of the tubal lesions to APSTs and noninvasive implants and their high frequency in these tubes that led to the current investigation.

**Johns Hopkins consultation cases—**The 7 consultation cases were sent to us around the same time as the analysis of the Danish cases was being performed. Although we have observed a number of such cases over the years, an exhaustive attempt to search for similar cases in our files was not undertaken.

### Definition of Papillary Tubal Hyperplasia

Because of the difficulty in the past in agreeing on what constitutes tubal hyperplasia[3,9,14,23] we restricted our definition to include only those tubal proliferations that exhibited papillary tufting and detached clusters of bland epithelium frequently, but not always, associated with psammona bodies. These clusters of epithelial cells and small papillae were found floating in the lumen or protruding from the tubal mucosa into the lumen and hence the lesion was termed “papillary tubal hyperplasia (PTH)”. As the number of papillae can vary considerably, for purposes of this study, we arbitrarily included only those cases in which, a minimum of 3 papillae/section were identified. Stratification of tubal cells alone was not sufficient for inclusion as this finding, which in some instances is probably due to tangential sectioning, is often found in fallopian tubes without any other evidence of hyperplasia and is therefore considered a normal variant.

## Results

### Microscopic Findings

Under low magnification the plical architecture has an abnormal, complex, “busy” appearance compared to the normal architecture (Figs. 1A and 1B). Under higher magnification the detached clusters of cells and small papillae floating in the tubal lumen are more clearly evident (Figs. 2–5). They are composed of a single layer of epithelium containing ciliated, secretory and intraepithelial lymphocytes, identical to the cellular composition of the normal tubal mucosa. The intraepithelial lymphocytes are located just above the basement membrane and are smaller than the ciliated and secretory cells. They contain a small, hyperchromatic, round or convoluted nucleus that is surrounded by a clear space or halo (Figs. 6,7). These cells have previously been termed “indifferent” cell and were thought to possibly be somatic stem cells of fallopian tube epithelium[22] but more recent studies have shown that they are lymphocytes which represent a form of mucosal-associated lymphoid tissue (MALT)[10]. Rare clusters of epithelial cells are flat and some contain a vacuole similar to that seen in ovarian serous tumors. Other clusters contain slightly larger cells with eosinophilic cytoplasm similar to eosinophilic metaplastic cells in the endometrium. Mitotic figures are rarely seen. Both the secretory and ciliated cells are bland and bear no resemblance to serous tubal intraepithelial carcinoma. As observed in our previous study[16] psammoma bodies can be found isolated in the tubal lumen or surrounded by a mantle of bland tubal epithelium in which case they qualify as “salpingoliths” (Figs. 5,6). In addition to the tubal lumen, psammoma bodies are also present in the epithelium and lamina propria of the tube (Fig. 6). A lesser degree of hyperplasia is characterized by small,

raised tufts of epithelium that emanate from the tubal epithelium (Fig. 8). As the hyperplasia evolves the tufts enlarge and form round clusters of cells identical to the clusters and papillae floating in the lumen but still attached to the tubal mucosa. Their connection to the epithelium is tenuous and gives the impression that they are about to be pinched off. (Figs. 7, 9). In some instances these attached small papillae contain a psammoma body in the core (salpingolith) identical to those papillae floating in the lumen. Due to the complex arrangement of tubal plicae, particularly in younger women, it is often difficult to be certain whether some of these papillary tufts without psammoma bodies are normal constituents of the tubal architecture cut tangentially or whether they are the earliest form of hyperplasia. In order to be conservative in determining the association of these lesions with APSTs, as indicated in the Materials and Methods section, we included only those in which there were at least 3 clusters/papillae per section. The presence of associated psammoma bodies was a useful feature in classifying a specimen as PTH as psammoma bodies are not a feature of normal fallopian tube epithelium.

The morphologic similarity of PTH, APSTs and noninvasive implants is striking (Fig. 10).

## Papillary Tubal Hyperplasia in Danish Cases

Patients ranged in age from 22–83 years with a mean of 42 years and a median of 41.5 years. A more detailed description of the demographic findings will be presented in a future publication that describes the clinical and pathologic features of the entire cohort. The women in the current study had a mean age approximately 10 years younger than the remainder of the cohort. In all other aspects they did not differ significantly.

**Tubal Findings—**(Table 1) As noted in the Materials and Methods section, in the Danish study a subset of cases with tubal implants were selected because adjacent tubal tissue was available for review. Papillary tubal hyperplasia was found in 20 (91%) of 22 cases and was not detected in 2 (9%). The 2 cases in which PTH was not detected had only one section in each case available for review. In 15 (75%) of the 20 cases with PTH, papillae were found in all the sections of tube examined whereas in 5 (25%) papillae were absent in some of the sections indicating that the process may not diffusely involve the tube. It appeared as if the ampullary portion of the tube was more frequently and more extensively involved compared to the infundibulum and isthmus, however, in this study the ampulla was more frequently sampled than the other parts of the tube. The number of intraluminal papillae varied from as few as 3 per case (two cases) to “too numerous to count” (6 cases). In the remaining cases the number of papillae ranged from 5–42. Psammoma bodies were found in 11 (50%) cases ranging from a few (2 per case) to many (too numerous to count). They were located in the lamina propria of the tubal plicae, epithelium and lumen. A chronic inflammatory infiltrate, confined to the lamina propria of the tube, was present in 8 (36%) of the 22 cases. One of these cases also demonstrated acute inflammation. Evidence of previous episodes of pelvic inflammatory disease based on agglutination of plicae, bridging of plicae across the tubal lumen or loss of plicae resulting in hydrosalpinyx was found in 8 (36%) of the 22 cases.

As mentioned in the Materials and Methods section all of the cases selected for study had implants involving the fallopian tube. They were noninvasive epithelial implants in 15 (68%) of the 22 cases, noninvasive desmoplastic implants in 4 (18%) and invasive implants (metastatic low-grade serous carcinoma) in 3 (14%). It should be noted that some noninvasive epithelial implants were surrounded by fibrotic tissue but they did not have the typical appearance of desmoplastic implants in which the epithelial component merges with the stromal component and has a mesothelial appearance. The 3 invasive implants were all associated with invasive low-grade serous carcinomas of the ovary.

**Associated Findings in the Ovary and Extraovarian Sites—**(Table 2) The associated ovarian tumor was an APST in 19 cases and an APST with an invasive low-grade serous carcinoma in 3. In addition to the fallopian tube, noninvasive implants were identified in the omentum in 2 cases and on the uterine serosa in one. Endosalpingiosis involving the omentum and tube was found in 6 (27%) of cases.

### Papillary Tubal Hyperplasia in Johns Hopkins Cases

The clinical and pathologic findings are summarized in Table 3. A detailed description of the 7 cases is included in an appendix.

Briefly, among the 7 JHH consultation cases the number of sections from the tubes ranged from 2–21. Papillary tubal hyperplasia was found in all sections in only two cases indicating that the lesion can be focal. Psammoma bodies were detected in 4 (57%) of the 7 cases. Chronic salpingitis was present in 3 (43%) of the cases, one of which was severe and also acute. In the latter case there was pyosalpinx and marked destruction of the plicae and in 2 other cases there was distortion of the plical architecture but no evidence of inflammation. Thus, there was evidence of pelvic inflammatory disease in 5 (71%) of the 7 cases. Endosalpingiosis was present in 4 (57%) cases and a noninvasive epithelial implant in another.

## Discussion

In 1969 Pauerstein and Woodruff wrote in the preface of their textbook on the fallopian tube "The era of indifference to the fallopian tube has passed"[22]. This statement was more prophetic than timely because it took almost another half a century for indifference to turn to intense interest as investigators proposed that intraepithelial carcinoma in the fallopian tube was the long sought for precursor of ovarian high-grade serous carcinoma[4,5,8,12,13,19]. As part of this renaissance in the role of the fallopian tube in ovarian carcinogenesis we renewed our interest in exploring the link between tubal hyperplasia and ovarian low-grade serous tumors.

Proliferative lesions in the fallopian tube other than carcinoma have received relatively little attention. There is general agreement that tubal hyperplasia exists but the criteria for its definition, including what constitutes the minimal degree of proliferation, have not been clarified[3,9,11,14,22,23]. Similarly, investigators have debated whether tubal hyperplasia is significantly associated with APSTs. Two studies that investigated this relationship arrived at diametrically opposite conclusions. Robey and Silva retrospectively reviewed representative sections of fallopian tubes from 99 women with SBTs and compared them to the fallopian tubes of 58 patients with cervical carcinoma and high-grade ovarian serous carcinoma and found tubal hyperplasia in 68.7% of the SBT cases compared to 25.9% in the control group ($p < 0.01$)[14]. In contrast, Yanai-Inbar and Silverberg analyzed fallopian tubes from 49 women with SBTs compared to a control group, which included other borderline tumors, a variety of invasive genital tract carcinomas, benign ovarian and uterine tumors and portions of tubes in women undergoing tubal ligation and found no significant association[23]. These authors graded tubal hyperplasia as mild, moderate and marked based on the degree of stratification and cellular atypia. They concluded that since mild hyperplasia was often found in women undergoing tubal ligation this degree of proliferation was within normal limits. Nonetheless, even moderate and marked hyperplasia showed no significant difference between the SBT cases and controls. Based on reviewing the criteria for tubal hyperplasia and the photomicrographs in these papers we believe that the different conclusions that were reached are due to the use of different criteria for the diagnosis of tubal hyperplasia.

Our analysis confirms that there are varying degrees of tubal hyperplasia. The earliest recognizable form begins with focal epithelial stratification followed by a slight uplifting of the tubal epithelium that forms a small tuft. With increasing proliferation the tuft expands and extends into the tubal lumen to form a small epithelial bud. The buds expand and form rounded papillae composed of cells identical to those in the tubal mucosa. The papillae can be solid in which case they are classified as “clusters” or they can contain a central stromal core in which case they are termed “papillae”. Since they appear to be variations on the same theme, we have used the terms interchangeably. As the tubal buds enlarge, they become more distinct, rounded and appear to be pinched off and extruded into the tubal lumen. Psammoma bodies can be present in the center of the papillae or are "naked" without associated epithelium. Besides the papillae in the tubal lumen, psammoma bodies are located in the lamina propria and the tubal mucosa. It is these papillae and psammoma bodies that are the key diagnostic features of PTH. The presence and location of the psammoma bodies is an intriguing aspect of this lesion as they are often the dominant feature. Their location in the lumen, epithelium and lamina propria suggest that they develop in the epithelium during the formation of an epithelial bud. It appears that the buds may then be either extruded into the tubal lumen or invaginate into the underlying stroma. The epithelium subsequently degenerates leaving a “naked” psammoma body in the tubal lumen or in the lamina propria. A similar process can occur in extratubal sites as well. In this study and in our experience it is not unusual to find a myriad of psammoma bodies coating peritoneal surfaces, notably the uterine serosa, ovaries and bowel serosa often with very little associated epithelium. We speculate that initially epithelium surrounded the psammoma bodies but then degenerated leaving only “naked” psammoma bodies which act as an irritant in the peritoneal cavity and induce fibrosis that can lead to adhesions and bowel obstruction.

The different conclusions reached by Robey and Silva and Yanai-Inbar and Silverberg regarding the association of ovarian serous borderline tumors with tubal hyperplasia probably relates to the different criteria that were used to diagnose tubal hyperplasia. Specifically, PTH is frequently associated with APSTs but lesser degrees of hyperplasia are not. Yanai-Inbar and Silverberg illustrate the very early budding stage (their Fig 3) but not the fully developed free floating epithelial clusters[21]. On the other hand Robey and Silva mention and illustrate the early budding stage as well as the detached luminal clusters of epithelial cells (their Fig 1) but they do not specifically indicate how often this latter change was found in their cases[14]. The findings in the current study strongly suggest that PTH is the most advanced stage of tubal hyperplasia and it is this lesion that is associated with APSTs. We speculate that lesser degrees of tubal hyperplasia may not necessarily progress to PTH and therefore are not significantly associated with APSTs. For that matter, not all PTH progresses to APSTs as illustrated by the 7 JHH cases in this study which were not associated with an ovarian tumor. It is conceivable that if the tubes in these cases were not removed PTH may have resulted in the development of an APST.

The fallopian tube has also been implicated in the development of endosalpingiosis. Sampson, who first described endosalpingiosis, believed that it arose from epithelium that was sloughed from the fallopian tubes[15]. Subsequently, Zinssner and Wheeler in a study of 128 omenta removed at autopsy and surgery reported 16 (14.8%) examples of endosalpingiosis[24]. Of further interest in their study was the presence of an associated serous cystadenoma in one case and SBTs in 3 others. One of these latter cases was reported as a serous carcinoma with metastases but the primary tumor and metastases were described as having numerous cilia and therefore based on this observation and their two photomicrographs (their Figs. 8 and 9) we interpret this as an SBT with noninvasive epithelial implants and endosalpingiosis. These authors found no transition of mesothelium to endosalpingiosis. They also noted that there were no examples of endosalpingiosis in the

male patients and therefore concluded that endosalpingiosis resulted from sloughing and implantation of tubal epithelium.

We postulate that PTH is responsible for the development of both endosalpingiosis and noninvasive epithelial implants even in the absence of an APSTs. Endosalpingiosis is composed of glands lined by a single layer of tubal-type epithelium. When endosalpingiosis exhibits papillary tufting or detachment of cell clusters or cribriform patterns and varying degrees of cytologic atypia it has been classified as “atypical endosalpingiosis”[2]. As the latter becomes more florid it merges into what is classified as a “noninvasive epithelial implant”. Endosalpingiosis, frequently accompanies SBTs and has been reported as high as 40% in some series[18]. In our Danish population-based study of low-grade serous tumors the prevalence of endosalpingiogsis was 10% (unpublished data). Accordingly, it appears that endosalpingiosis and noninvasive epithelial implants are part of a continuum of benign low-grade serous proliferations involving the peritoneum that result from implantation of tubal epithelium. Although sometimes noninvasive epithelial implants are embedded in fibrous tissue, they differ from noninvasive desmoplastic implants, morphologically and immunohistochemically (unpublished data). The latter typically display a biphasic epithelial and spindle cell growth pattern often associated with a chronic inflammatory infiltrate. It is conceivable that some of these may develop in situ. In contrast, invasive implants are metastatic low-grade serous carcinoma from either a noninvasive low-grade ovarian serous carcinoma (micropapillary serous) or an invasive low-grade serous carcinoma[19]. In the current study the three cases of invasive implants were all associated with an ovarian low-grade serous carcinoma.

Another interesting observation in this study, as in our previous study on salpingoliths[16], was the frequent association of chronic salpingitis and distorted tubal architecture consistent with previous pelvic inflammatory disease with PTH and endosalpingiosis. Zinsser and Wheeler in their autopsy study reported 14 (88%) of 16 cases of endosalpingiosis had evidence of chronic salpingitis either in the form of chronic inflammation or distorted tubal architecture[24]. It is well known that inflammation may stimulate proliferation of tubal epithelium and therefore it is plausible that chronic salpingitis may play a role in the pathogenesis of PTH and endosalpingiosis. In the current study evidence of pelvic inflammatory disease in the form of chronic salpingitis or distorted tubal architecture was found in 35–70% of the cases (Danish and JHH cases respectively).

Three possible mechanisms can explain the association of PTH and APSTs. One is that the tubal and ovarian lesions arise independently, a process that has been termed a “field effect”. A second is that the ovarian tumor is primary and that the clusters of cells and papillae in the tubal lumen are a secondary phenomenon. The third possibility is that the tubal lesion is primary and that the ovarian and extraovarian lesions (APST, noninvasive epithelial implants and endosalpingiosis) are secondary. Robey and Silva concluded that tubal hyperplasia and SBTs developed independently as a result of a so-called "field effect"[14] as did McCaughey et al. in their study of SBTs and implants[7]. This implies that SBTs develop from peritoneal mesothelium, through a process of metaplasia resulting in their müllerian phenotype. The concept of müllerian metaplasia of peritoneal mesothelium, although difficult to completely disprove has, on the other hand, no evidence to support it. In the current study, the morphologic similarity of PTH, noninvasive epithelial implants and APSTs is striking. All of these lesions, in addition to being composed of ciliated and secretory cells identical to those in normal fallopian tube epithelium, also contain intraepithelial lymphocytes which are also found in normal tubal epithelium. Invoking metaplasia of the peritoneum (mesothelium) to account for the development of this complex assortment of cells seems highly unlikely. In addition, in our experience PAX 8, a müllerian marker, is expressed in fallopian tube epithelium and APSTs whereas calretinin, a

mesothelial marker, is not. In contrast, the ovarian surface epithelium strongly expresses calretinin but not PAX 8 (unpublished data). Furthermore, psammoma bodies are an integral part of PTH as they are found in the majority of cases and psammoma bodies are frequently associated with APSTs. In contrast, psammoma bodies are typically not associated with mesothelial proliferations and when they are, they usually are few in number. Finally, noninvasive epithelial implants, like endosalpingiosis, have not been reported in male patients. Not withstanding rare reports of endometriosis in the prostate of men treated with estrogen[1] there is no support for the argument that female sex steroid hormones are responsible for müllerian metaplasia of the peritoneum leading to the development of endosalpingiosis and noninvasive epithelial implants in women. We believe the most plausible explanation is that PTH is the precursor of all of these lesions since it was identified in 7 cases in the current study in the absence of a primary ovarian tumor. Moreover, in one of these cases, a noninvasive epithelial implant was identified and in 4 cases PTH was associated with endosalpingiosis.

Based on the findings in this study we propose the following model for the origin and development of the entire spectrum of pelvic low-grade serous proliferations. Chronic inflammation induces a proliferation of tubal epithelium that can progress to PTH in some women. Epithelial clusters and papillae are shed and implant on ovarian and peritoneal surfaces. On the ovary the lesion is termed “cortical inclusion cyst” and when it involves extraovarian sites it is termed “endosalpingiosis”. In contrast to cortical inclusion cysts lined by tubal-type epithelium, some cysts are lined by flat epithelium and represent invaginated clefts from the surface of the ovary that have been tangentially cut. Accordingly, there are two types of cortical inclusion cysts, one derived from invaginated surface epithelium and therefore of mesothelial origin and another resulting from implanted tubal epithelium and therefore of müllerian origin. It is also conceivable that normal tubal epithelium can implant directly on the ovary at the time of ovulation when the ovarian surface epithelium is disrupted and the fimbria are in direct contact with the denuded ovarian surface. When endosalpingiosis exhibits a complex glandular arrangement with varying degrees of cytologic atypia it qualifies for the diagnosis of “atypical endosalpingiosis” which merges imperceptibly into noninvasive epithelial implants. If mutation of *KRAS* or *BRAF* occurs in any of these lesions an APST develops[20]. This model can explain the development not only of ovarian APSTs but also the development of noninvasive implants and APSTs involving pelvic and abdominal sites in the absence of an ovarian tumor.

As previously noted, one of the aims of our ongoing population-based study of ovarian low-grade serous tumors in Denmark is to identify biomarkers to assist in the distinction of noninvasive and invasive implants and therefore the presence of PTH was not recorded in the data collection. We are, therefore, unable to determine the frequency of PTH in the study population. In an effort to address this issue we are currently performing a study comparing the frequency of PTH in women with APSTs to a control group of patients in whom the entire fallopian tube has been processed using the SEE-FIM technique[8] to determine more accurately the frequency of this lesion in women with APSTs and in the normal population.

In conclusion, we propose a model for the development of all low-grade serous proliferations involving ovarian and extraovarian sites (APSTs, noninvasive epithelial implants and endosalpingiosis). The process begins with chronic inflammation leading to tubal hyperplasia, which if it progresses to PTH can shed and implant tubal epithelium on ovarian and peritoneal surfaces resulting in a variety of low-grade serous proliferations. If this hypothesis is confirmed it would indicate that all ovarian serous tumors, low- and high-grade, originate from tubal epithelium and involve the ovary secondarily.

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## Appendix

Case #1 A 44-year-old woman presented with pelvic pain, pressure, menorrhagia and symptomatic fibroids. She underwent a supracervical hysterectomy and bilateral salpingectomy. At surgery her ovaries were noted to be normal. Gross examination disclosed uterine leiomyomas up to 3.1 cm. in greatest dimension. The fallopian tubes (sides not separately designated) measured 5 cm in length and 0.6 cm in diameter. Microscopic examination confirmed the uterine leiomyomas. There were a total of 21 sections from both tubes. Multiple small intraluminal papillae and psammoma bodies (too numerous to count) were identified in 9 sections and not in 2. The other 10 sections could not be properly evaluated because of extensive cautery artifact. A chronic inflammatory infiltrate was not present but the plicae were fibrotic, some bridging across the tubal lumen indicative of previous episodes of salpingitis. Endosalpingiosis was present on the uterine serosa in one section and also on the serosa of one of the fallopian tubes. A noninvasive epithelial implant was present on the serosa of the fallopian tube in another section.

Case #2 A 65-year-old woman presented with postmenopausal bleeding and underwent a total abdominal hysterectomy and bilateral salpingo oophorectomy. Peritoneal washings were obtained. On gross examination the ovaries were almost identical in size measuring 3×2.5×1 cm. The fallopian tubes were neither measured nor described. A 1.8 cm polyp was present in the endometrium. Microscopic examination confirmed the presence of a benign endometrial polyp. In addition there was a well-differentiated mucinous endometrial carcinoma invading the myometrium (depth of invasion was not indicated on the accompanying surgical pathology report and could not be determined based on the submitted sections). Large numbers of psammoma bodies enmeshed in fibrous adhesions were present on the uterine serosa. Small foci of endosalpingiosis were also present. The surface of the ovaries was similarly coated with psammoma bodies embedded in fibrous adhesions as well as containing foci of endosalpingiosis. Numerous cortical inclusion cysts lined by tubal epithelium were present as well as a serous adenofibroma on the surface of one ovary. A total of 4 sections from both fallopian tube were available for review and contained numerous intraluminal papillae and psammoma bodies in 3 sections (both sections involved). In one section numerous psammoma bodies and tiny clusters of epithelium that barely qualified for the diagnosis of endosalpingiosis were present on the tubal serosa. A mild chronic inflammatory infiltrate was present in the lamina propria of the plicae, which were markedly distorted, thickened, fibrotic, clubbed and absent in the center of the lumen in some sections. The peritoneal fluid contained psammoma bodies and clusters of epithelium similar to those in the tubal lumen.

Case #3 A 52-year-old G2, P2002 woman was admitted for surgical evaluation following the diagnosis of a low-grade papillary serous carcinoma in a hernioraphy specimen (slides not available for review) 3 months earlier. She underwent exploratory laparotomy, lysis of adhesions, total omentectomy, supracervical hysterectomy and bilateral salpingo

oophorectomy. Surgical findings included a thickened omentum and extensive adhesions. There was no other evidence of disease in the abdomen, pelvis and ovaries.

On gross examination there were adhesions on the uterine serosa and surface of the ovaries. The right ovary measured 4×1.5×1.5 cm and left ovary 4×2×2.5 cm. The fallopian tubes appeared normal and measured 5.5 cm in length and 0.8 cm. On microscopic examination the omentum was riddled with psammoma bodies; scattered foci of endosalpingiosis were also present. The surface of the ovaries and uterine serosa was covered by adhesions containing large numbers of psammoma bodies and scattered foci of endosalpingiosis. A total of 4 sections from both fallopian tubes were available for review; numerous intraluminal papillae and psammoma bodies were found in all of them. There was no evidence of inflammation and the plicae were not significantly distorted.

Case #4 A 49-year-old woman G1P0010 was admitted with a history of an abnormal Pap smear and an endometrial biopsy diagnosed as simple hyperplasia with no atypia. Imaging studies revealed a 4 cm ovarian cyst. CA125 was 12. She underwent a total abdominal hysterectomy and bilateral salpingo oophorectomy. At surgery the uterus and tubes were described as normal. The left ovary was normal. The right ovary measured 4 cm and contained a simple cyst. A few adhesions were present. On gross examination the uterus appeared normal as did the ovaries and fallopian tubes. The right ovary measured 4×3.5×3.5 cm and the left ovary 3.5×1.5×2 cm. The right fallopian tube measured 6.5 cm in length and 0.7 cm in diameter. The left fallopian tube measured 5.5 cm in length and 0.6 cm in diameter. Microscopically, 2 sections from the left fallopian tube were available and both contained a large number of intraluminal papillae but no psammoma bodies. Slides from the right fallopian tube were not available for review. Two outside pathologists had classified the lesion as tubal papillary carcinoma. There was no evidence of inflammation but tubal plicae were blunted and bridged across the lumen resulting in abnormal architecture.

Case #5 A 60-year-old woman underwent a total abdominal hysterectomy and bilateral salpingo oophorectomy and omentectomy for endometrial carcinoma. Aside from numerous small, miliary nodules noted in the omentum there were no other unusual findings. On gross examination a 2 × 2 cm nodular mass was present in the endometrium. The right fallopian tube measured 2.5 × 0.5 cm and the right ovary was nodular and measured 3.5 × 2 × 1 cm. The left tube measured 3.5 × 0.5 cm and the left ovary measured 2.5 × 2.5 by 1 cm. The omentum measured 25 × 13 ×2 cm and contained small, scattered white firm areas. Nine sections of fallopian tube were available for microscopic review. Chronic salpingitis was present but the tubal architecture was not distorted. A very large number (too numerous to count) of small papillae and epithelial cell clusters associated with psammoma bodies was identified in 7 of the 9 sections (both sides). The papillae were located in the tubal lumen and attached to or were budding from the tubal mucosa. A myriad of psammoma bodies was present predominantly in the tubal lumen but also in the tubal epithelium and the lamina propria. Psammoma bodies were either surrounded by a single layer of tubal epithelium or were completely devoid of epithelium. Psammoma bodies alone were the predominant finding in the omentum where they were associated with dense fibrotic tissue. Very little epithelium was present, barely qualifying for the diagnosis of endosalpingiosis. The right ovary was encased by a multinodular adenofibromatous proliferation. These lesions were composed almost entirely of dense fibrotic tissue surrounded by a single layer of bland tubal-type epithelium. They did not form a discrete mass and were therefore not interpreted as a tumor. The uterine carcinoma was a high-grade endometrioid carcinoma with serous features. There was no myoinvasion or cervical involvement.

Case #6 A 45-year old woman with a prior unilateral salpingectomy was admitted for evaluation of dysmenorrhea and abnormal uterine bleeding and for sterilization.

Laparoscopy was performed which revealed moderate endometriosis involving the cul de sac, uterosacral ligaments bilaterally and the anterior bladder flap. These lesions were fulgurated. A uterine fibroid was also noted. The ovaries were normal. A unilateral salpingectomy was performed. On gross examination the tube measured 6 cm in length and 0.5–0.75 cm in diameter and was otherwise unremarkable. Four sections of fallopian tube were available for microscopic review but 2 were severely and one partially cauterized so that complete evaluation was possible on only one section. In this section approximately 30 papillae were floating in the tubal lumen or were minimally attached to the tubal mucosa. There were no psammoma bodies nor was there evidence of distortion of the plical architecture or salpingitis.

Case # 7 A 56-year-old woman was admitted for a total abdominal hysterectomy and bilateral salpingo oophorectomy for treatment of endometrial carcinoma. Gross examination revealed a 6 cm granular mass filling the endometrial cavity that involved the anterior and posterior surfaces. The left fallopian tube was dilated, swollen and exuded cloudy, mucoid fluid on sectioning. It was 2.5 cm in diameter. The left ovary measured 2.5 cm and was unremarkable. The right tube measured 7 cm in length and 0.8 cm in diameter. The right ovary measured 2.5 × 2.0 × 1.8 cm and was unremarkable. Microscopically, the uterine tumor was a poorly differentiated endometrioid carcinoma with squamous differentiation invading the inner half of the myometrium. Four sections of the left tube were available for microscopic review. Small papillae that were too numerous to count completely filled the tubal lumen in 2 sections; psammoma bodies were not identified. None were identified in the other 2 sections. In addition to the papillae, very tiny clusters composed of 2 or 3 cells and even individual cells were present in the lumen in very large numbers. There was marked acute and chronic salpingitis and pyosalpinx with destruction of tubal plicae. Only one partial section of tube was available from the right side and it was unremarkable. Aside from periovarian adhesions on the left ovary, the ovaries were unremarkable.

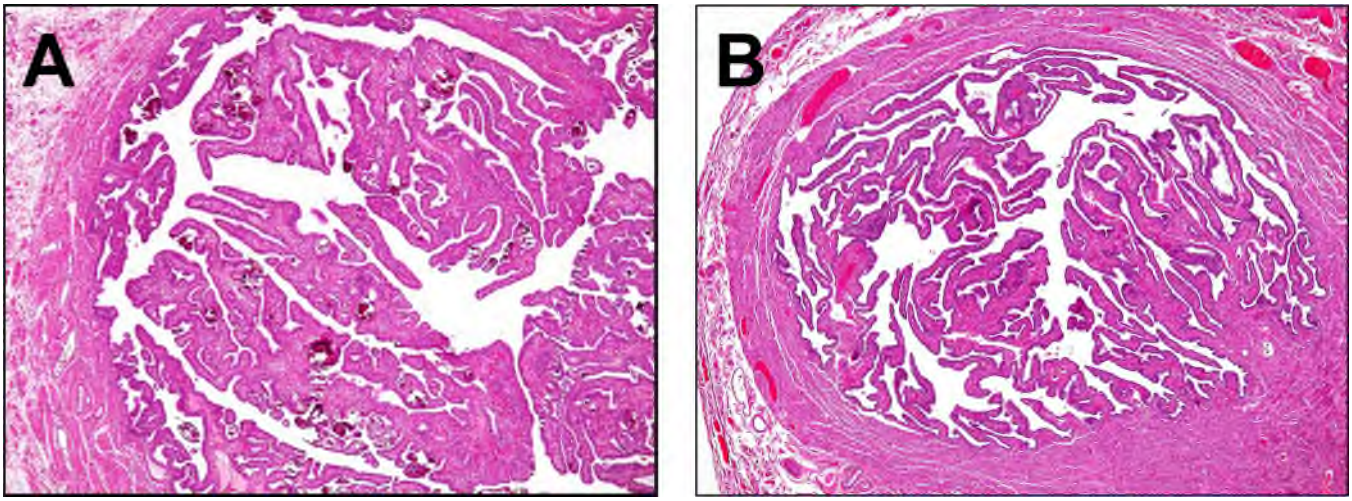


**Fig. 1.**

A Papillary tubal hyperplasia. Although the overall plical architectural changes are subtle, the plicae are slightly thicker and the overall appearance looks “busy”.

B Normal fallopian tube.

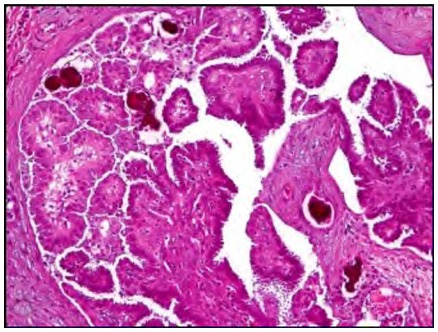

**Fig. 2.**
Papillary tubal hyperplasia. Numerous small papillae and psammoma bodies in the tubal lumen.

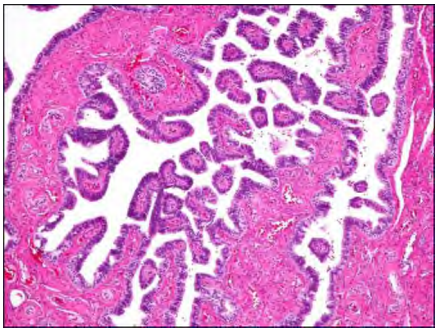

**Fig. 3.**
Papillary tubal hyperplasia. Multiple small papillae floating in tubal lumen.

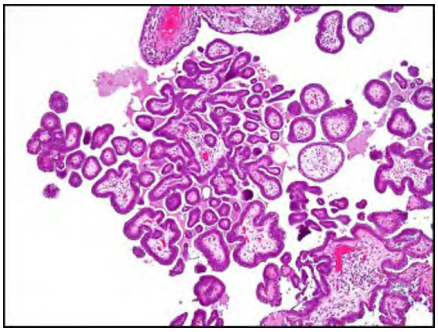

**Fig. 4.**
Papillary tubal hyperplasia. A profuse number of small papillae in the tubal lumen.

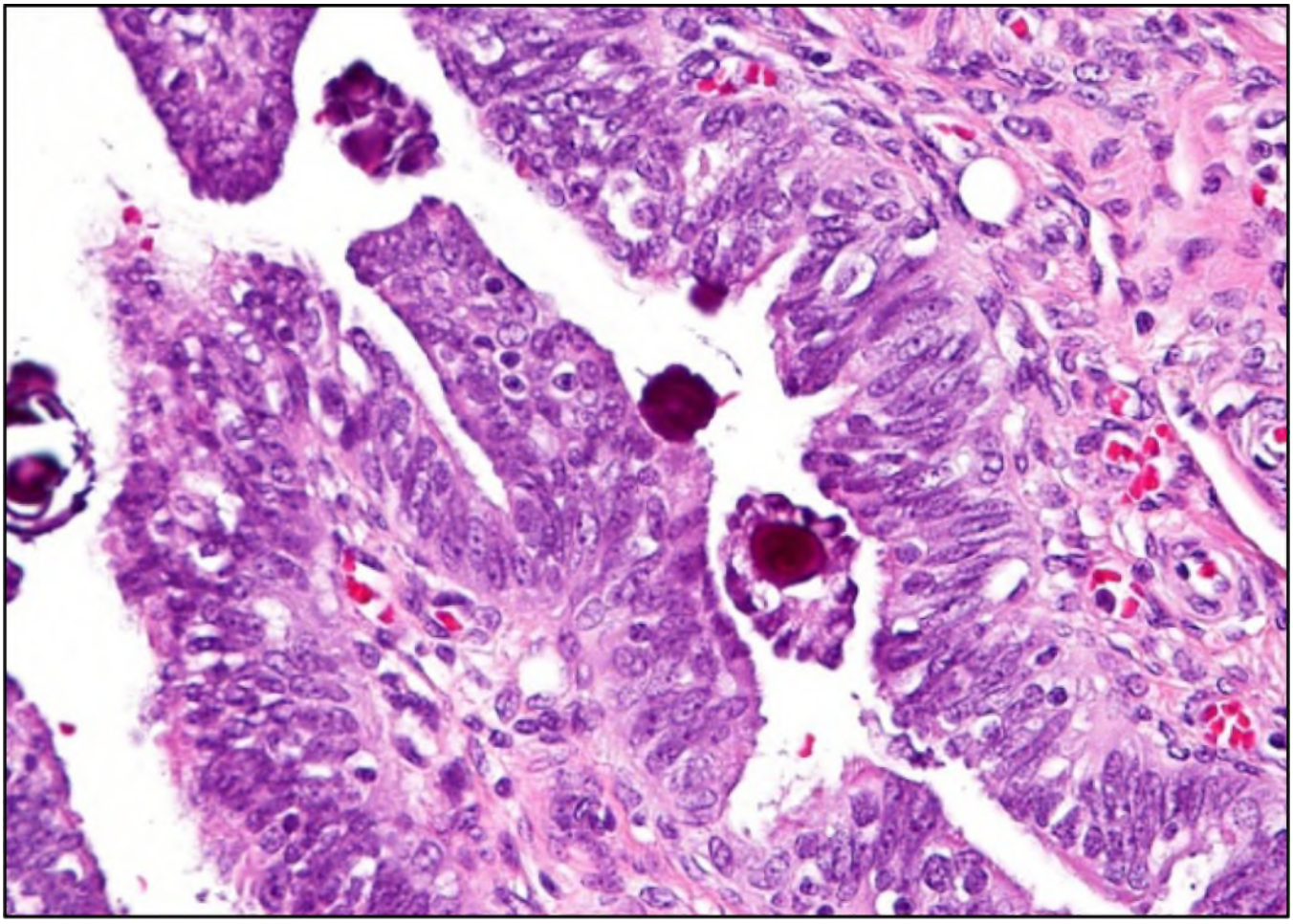

**Fig. 5.**
Papillary tubal hyperplasia. A “naked” psammoma body lying on surface of tubal epithelium and another one within the core of a small papilla, so-called salpingolith.

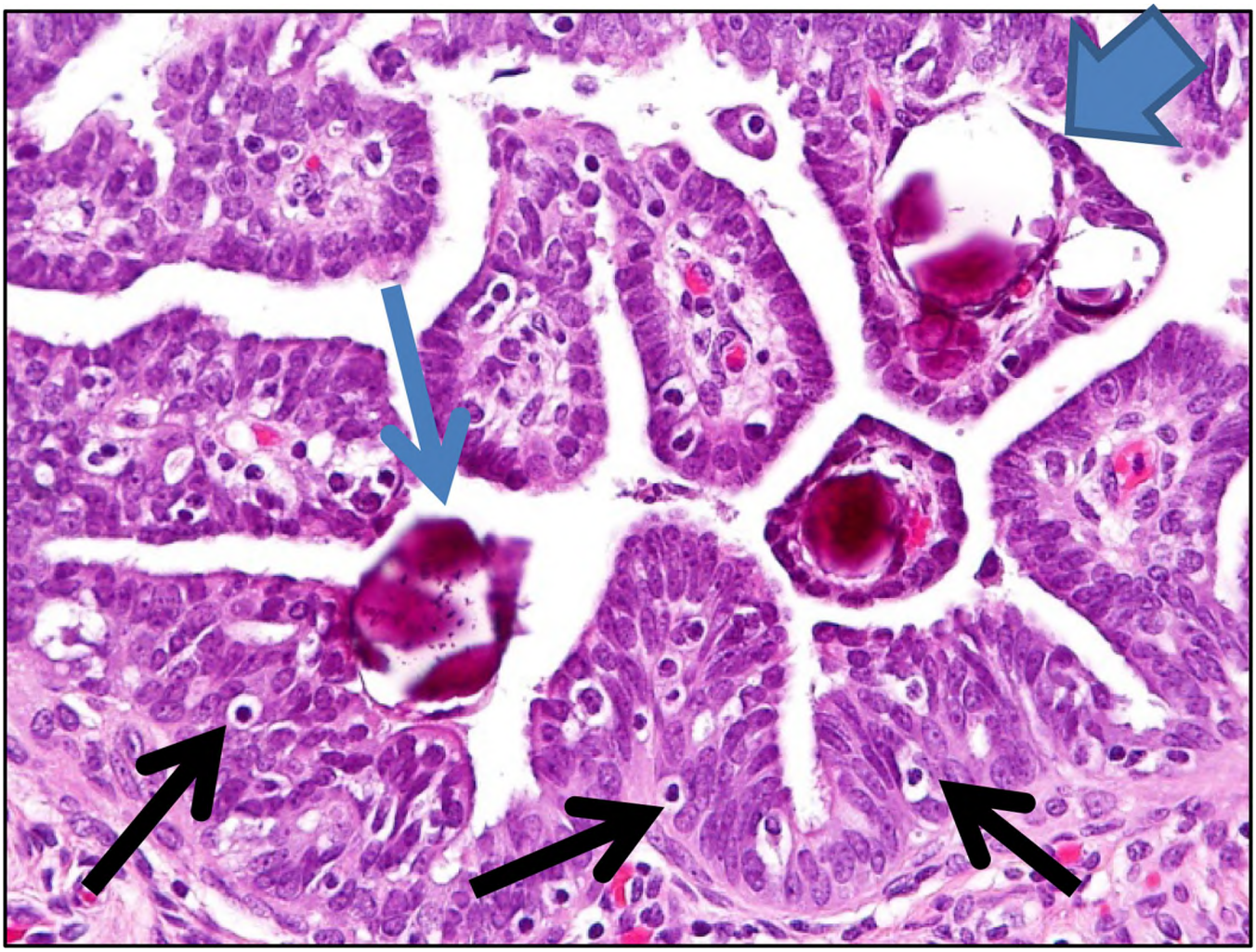

**Fig. 6.**

Papillary tubal hyperplasia. Psammoma bodies within a papilla (salpingolith), tubal epithelium (blue thin arrow) and lamina propria (blue block arrow). Numerous intraepithelial lymphocytes are located just above the basement membrane in the mucosa (black arrows).

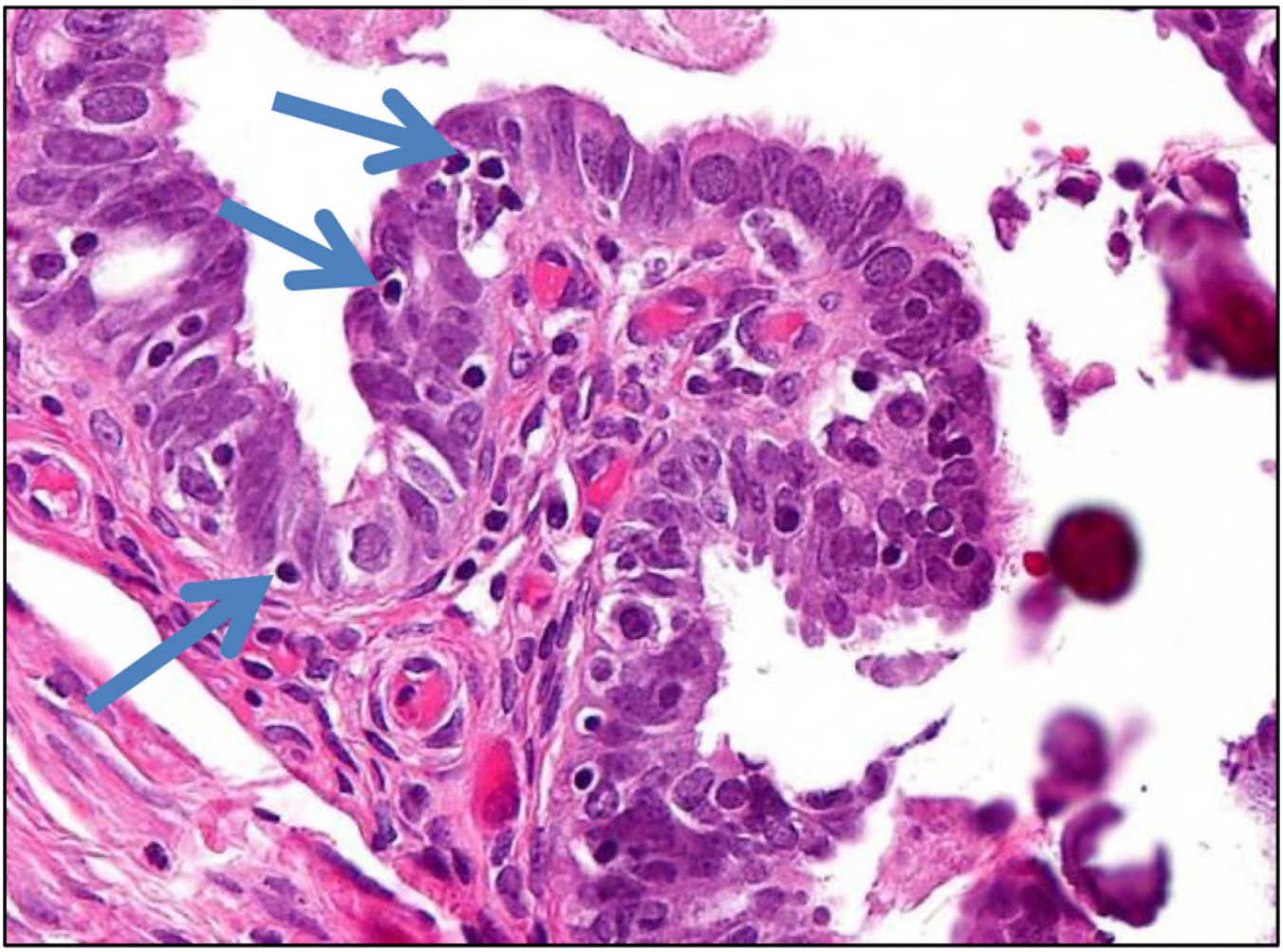

**Fig. 7.**

Papillary tubal hyperplasia. Papilla arising from mucosa before it becomes pinched off. Secretory cells lie between ciliated cells. Intraepithelial lymphocytes lie just above the basement membrane (arrows). The nucleus is characteristically surrounded by a halo.

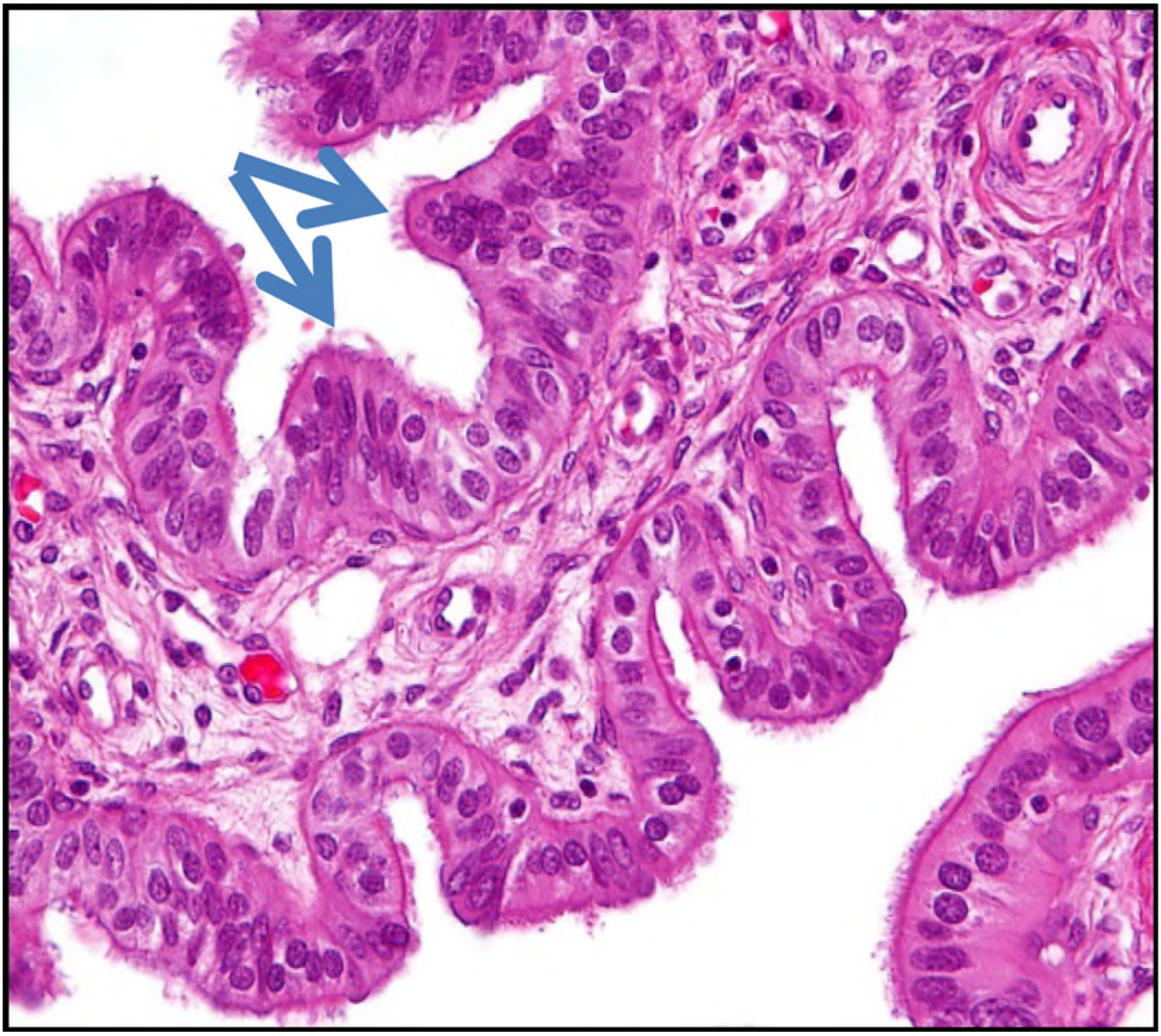

**Fig. 8.**
Early tubal hyperplasia. Small elevated tufts of epithelium (arrows) represent the earliest hyperplastic change.

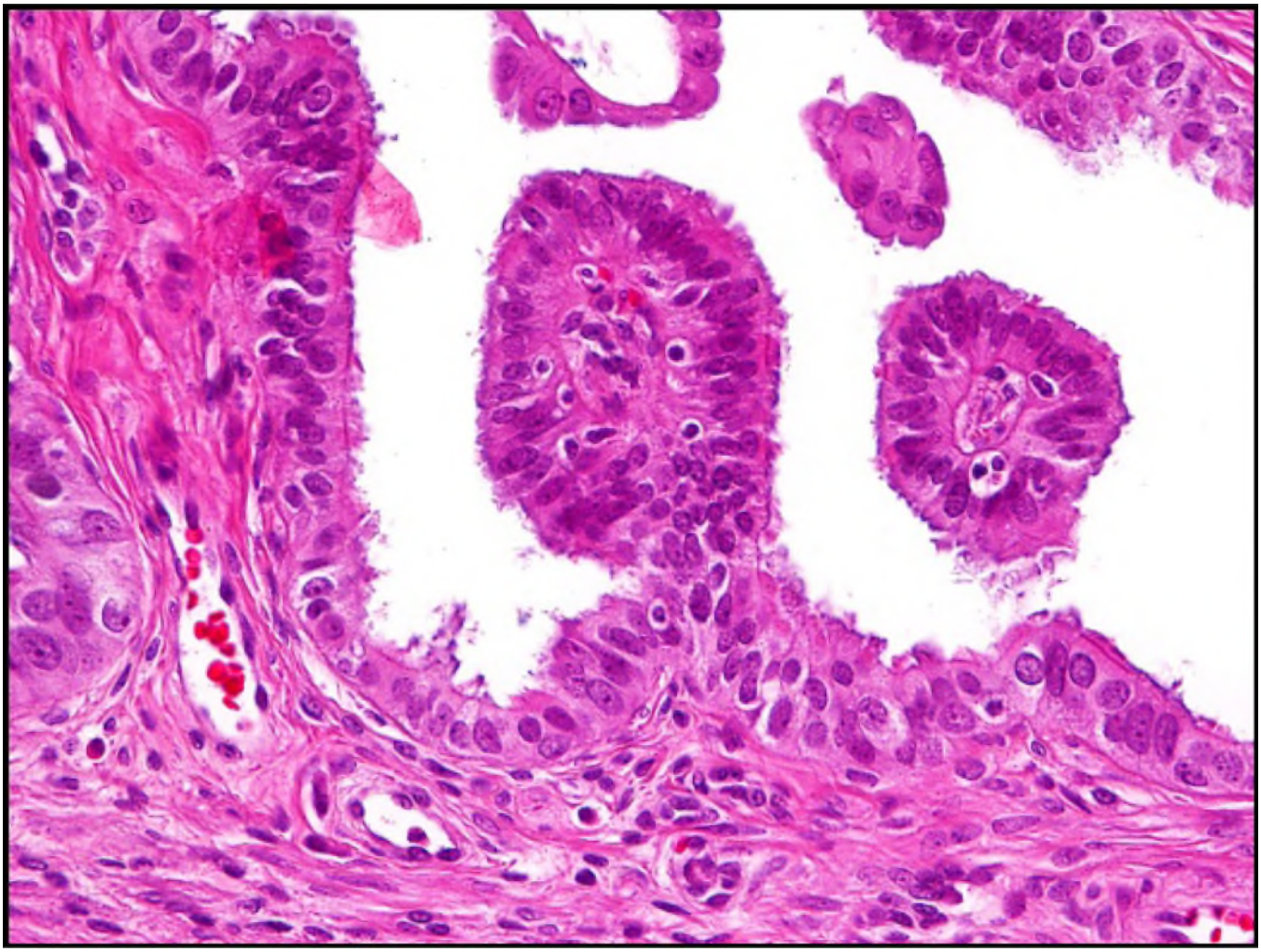

**Fig. 9.**

Early tubal hyperplasia. Papillary bud still attached to tubal mucosa appears to be the next stage of hyperplasia before being pinched off and floating into the tubal lumen.

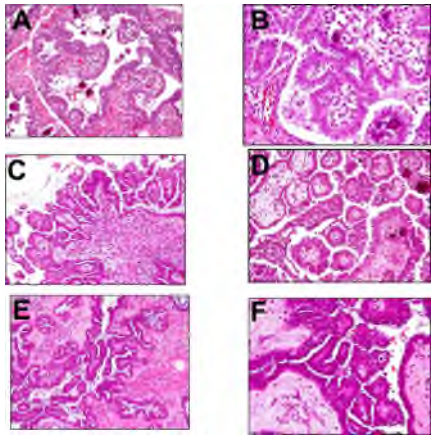


**Fig. 10.**

A and B. Papillary tubal hyperplasia; C and D. Noninvasive epithelial implant; E and F. Atypical proliferative (borderline) serous tumor. Images are from different cases.

**Table 1**

Patient age and Fallopian Tube Findings in Danish Cases (n=22)

| Case No. | Age Years | No. PTH/ Total Tube Sections | No. Papillae in Lumen | Psammoma Bodies | Features of Salpingitis |
|---|---|---|---|---|---|
| 1 | 83 | 1/2 | 3 | None | No |
| 2 | 22 | 1/3 | 10 | None | Yes |
| 3 | 33 | 2/2 | TNC | Yes | Yes |
| 4 | 40 | 3/4 | 20 | No | Yes |
| 5 | 25 | 1/1 | 12 | Yes | Yes |
| 6 | 34 | 2/2 | 42 | Yes | Yes |
| 7 | 55 | 1/1 | TNC | None | No |
| 8 | 51 | 1/1 | 12 | Yes | No |
| 9 | 33 | 2/2 | 20 | Yes | No |
| 10 | 52 | 1/1 | 5 | None | No |
| 11 | 43 | 2/2 | 8 | None | No |
| 12 | 32 | 1/1 | 15 | None | No |
| 13 | 36 | 0/1 | None | None | No |
| 14 | 41 | 1/1 | TNC | Yes | Yes |
| 15 | 48 | 1/1 | TNC | Yes | No |
| 16 | 51 | 2/2 | TNC | Yes | Yes |
| 17 | 43 | 1/1 | 4 | None | Yes |
| 18 | 61 | 1/3 | 5 | Yes | No |
| 19 | 55 | 2/2 | TNC | Yes | No |
| 20 | 33 | 2/2 | 3 | Yes | No |
| 21 | 25 | 0/1 | None | None | No |
| 22 | 41 | 2/4 | 9 | None | No |

Abbreviations: PTH= papillary tubal hyperplasia, TNC= too numerous to count

*Am J Surg Pathol*. Author manuscript; available in PMC 2012 November 1.

**Table 2**

Extratubal Findings in Danish Cases (n=22)

| Case # | PTH | Ovarian Tumor | Noninvasive Implants | Invasive Implants | Endosalpingiosis |
|---|---|---|---|---|---|
| 1 | Yes | APST/LGSC | No | Yes | No |
| 2 | Yes | APST | Yes | No | No |
| 3 | Yes | APST | Yes | No | No |
| 4 | Yes | APST | Yes | No | Yes |
| 5 | Yes | APST | Yes | No | No |
| 6 | Yes | APST | Yes | No | No |
| 7 | Yes | APST | Yes | No | No |
| 8 | Yes | APST | Yes desmoplastic | No | No |
| 9 | Yes | APST | Yes | No | No |
| 10 | Yes | APST/LGSC | No | Yes | No |
| 11 | Yes | APST | Yes | No | Yes |
| 12 | Yes | APST | Yes | No | No |
| 13 | No | APST | Yes | No | No |
| 14 | Yes | APST | Yes | No | Yes |
| 15 | Yes | APST | Yes desmoplastic | No | No |
| 16 | Yes | APST | Yes desmoplastic | No | Yes |
| 17 | Yes | APST | Yes desmoplastic | No | No |
| 18 | Yes | APST | Yes | No | Yes |
| 19 | Yes | APST | Yes | No | No |
| 20 | Yes | APST/LGSC | No | Yes | No |
| 21 | No | APST | Yes | No | Yes |
| 22 | Yes | APST | Yes | No | No |

Abbreviations: APST= atypical proliferative serous tumor, LGSC= low-grade serous carcinoma, PTH= papillary tubal hyperplasia

**Table 3**

Clinicopathologic Findings in Johns Hopkins Consult Cases (n=7)

| Consult case No. | Age years | Indication for surgery | Psammoma Bodies in FT | Features of salpingitis | Endosalpingiosis | Noninvaisive implant |
|---|---|---|---|---|---|---|
| 1 | 44 | leiomyoma | yes | yes | yes | yes |
| 2 | 65 | Postmenopausal Bleeding | yes | yes | yes | no |
| 3 | 52 | Exploratory laparotomy for Dx of carcinoma* | yes | no | yes | no |
| 4 | 49 | Endometrial hyperplasia | no | yes | no | no |
| 5 | 60 | Endometrial carcinoma | yes | yes | yes | no |
| 6 | 45 | Endometriosis | no | no | no | no |
| 7 | 56 | Endometrial carcinoma | no | Present pyosalpinx | no | no |

Abbreviations: Dx= diagnosis, FT= fallopian tube

*Low-grade papillary serous carcinoma diagnosed in a hernia specimen at an outside hospital. Slides not available for review.

# Exhibit E

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# ON TALC TRANSLOCATION FROM THE VAGINA TO THE OVIDUCTS AND BEYOND*

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(*Received* 9 *July* 1985)

**Abstract**—The objective of this study was to investigate whether multiple vaginal depositions of neutron-activated talc in the cynomolgus monkey result in the translocation of this material to the uterus and beyond. Within a 45-day period, six monkeys received 30 applications of 125 mg neutron-activated talc, suspended in 0.3 ml physiological saline solution containing 1% carboxymethyl cellulose as a suspending agent. The suspension was deposited in the posterior vaginal fornix of the sedated monkeys. Two days after the final talc application, the animals were anaesthetized. Abdominal lavage was performed and the lavage fluid collected for $\gamma$-ray analysis. Also collected for $\gamma$-ray analysis were the following tissues/organs: ovaries, oviducts, uterus, and vagina with cervix. Six untreated control monkeys underwent the same procedures. The radioisotopes $^{46}Sc$, $^{60}Co$, $^{59}Fe$ and $^{51}Cr$ in the activated talc served as tracers. Only the samples containing vagina and cervix from the dosed monkeys contained varying quantities of talc. This demonstrates that no measurable quantities of talc, deposited by multiple applications in the vaginal fornix of the cynomolgus monkey, translocated to the uterus or beyond.

## INTRODUCTION

Ever since Egli & Newton (1961) reported the apparent translocation of carbon black from the vagina to the oviducts in two of three female patients, increasing interest has focused on the question of whether particles can, indeed, migrate from the vagina to the oviducts and beyond. This question received additional attention following the observations of Henderson, Joslin, Turnbull & Griffiths (1971), who reported talc particles in 10 of 13 ovarian tumours in humans. These findings imply a translocation of talc from the vagina to the ovaries. Talc can be deposited in the vagina by dusting the perineum, or from sanitary napkins, diaphragms or condoms.

The results of several subsequent studies (DeBoer, 1971; Gardner, Fink & Hassler, 1980; Hassler, Gardner, Emmerling *et al.* 1974; Venter & Itteralde, 1979) were, in part, ambiguous (see under Discussion). Whether "insoluble", inanimate particles, deposited in the vagina, can penetrate the cervical barrier and migrate "upstream" against the ciliary beat of the oviductal epithelium without the aid of manipulative forces remained to be conclusively demonstrated.

In a pilot study (Wehner, Hall, Weller *et al.* 1985) prior to the more definitive study described in this paper, we first attempted to reproduce the results of Egli & Newton (1961) in the cynomolgus monkey (*Macaca fascicularis*), following their procedures as closely as practical. While our results suggested that no translocation of bone black particles took place, translocation could not be ruled out with certainty in the absence of quantitative analyses. Results of a quantitative experiment in the monkeys, for which we used neutron-activated talc to circumvent the problem of environmental contamination, indicated that no measurable quantities ($> \sim 0.5\ \mu g$) of talc translocated from the deposition site in the vagina to the uterine cavity and beyond (Wehner *et al.* 1985). However, to be more conclusive, our results needed to be reproduced in a larger number of animals following multiple applications.

## EXPERIMENTAL

A purified blend of cosmetic talc, supplied by the Cosmetic, Toiletry and Fragrance Association, Inc., and appropriate standards were exposed for 6.5 hr to an estimated neutron fluence of $1.2 \times 10^{17}$ n/cm$^2$ in a 1 megawatt TRIGA reactor at Washington State University.

Using the detector efficiency curve generated when the neutron flux was determined, the talc was characterized in terms of disintegrations per minute (dpm) per $\mu$g talc (Table 1). Using a United States Geological Survey BHVO standard as a comparative standard, elemental concentrations in the talc sample were determined (Table 2). Counting time for the talc characterization was 20,000 seconds/sample (5.5 hr).

A quantity of neutron-activated talc was suspended in physiological saline solution containing 1% carboxymethyl cellulose (CMC; Sigma Chemical

*This work was performed by Battelle, Pacific Northwest Laboratories for the Cosmetic, Toiletry and Fragrance Association, Inc., Under Contract No. 2311205966.

Table 1. Radionuclide concentrations in talc sample

| Radionuclide | Concentration* |
|---|---|
| $^{141}$Ce | 0.018 ± 3.2 |
| $^{58}$Co | 0.0839 ± 1.0 |
| $^{60}$Co | 0.297 ± 0.9 |
| $^{51}$Cr | 2.29 ± 0.9 |
| $^{59}$Fe | 0.617 ± 0.7 |
| $^{177}$Lu | 0.093 ± 9.3 |
| $^{54}$Mn | 0.026 ± 2.1 |
| $^{124}$Sb | 0.0039 ± 11.7 |
| $^{46}$Sc | 0.316 ± 0.7 |
| $^{169}$Yb | 0.010 ± 12 |
| $^{65}$Zn | 0.015 ± 6.9 |

*Given as dpm/μg talc ± 1σ counting error (error expressed as a percentage).

Table 2. Elemental concentration in the talc sample

| | | USGS BHVO | |
|---|---|---|---|
| Element | Concentration* | Counted* | Stated standard |
| Scandium | 1.02 ± 0.4 | 30.0 ± 0.3 | 30 |
| Chromium | 117 ± 0.8 | 290 ± 1.0 | 290 |
| Iron | 9780 ± 0.6 | 85200 ± 0.6 | 85100 |
| Cobalt | 20.7 ± 0.8 | 45.0 ± 1.0 | 45 |
| Nickel | 394 ± 20 | 120 ± 28 | 120 |
| Zinc | 15 ± 10 | 102 ± 10 | 102 |
| Antimony | 0.015 ± 7.9 | 0.17 ± 7.2 | 0.17 |
| Cerium | 3.97 ± 3.3 | 40.0 ± 3.1 | 40 |
| Europium | 0.084 ± 4.9 | 2.1 ± 3.4 | 2.1 |
| Terbium | 0.087 ± 18 | 1.0 ± 16 | 1.0 |
| Lutetium | 0.037 ± 12 | 0.32 ± 12 | 0.32 |
| Hafnium | 0.19 ± 7.7 | 4.10 ± 4.4 | 4.1 |
| Tantalum | 0.071 ± 13 | 1.08 ± 12 | 1.08 |
| Thorium | 0.35 ± 13 | 1.0 ± 18 | 1.0 |
| Ytterbium | 0.28 ± 14 | 2.1 ± 16 | 2.1 |

USGS BHVO = United States Geological Survey
*Given as ppm ± 1σ counting error (error expressed as a percentage).

Co., St Louis, MO) as a suspending agent so that 0.3 ml of the suspension contained 125 mg talc.

From 12 female exbreeder cynomolgus monkeys, obtained from the Medical Lake Field Station of the Regional Primate Research Center at the University of Washington, six monkeys with the most regular menstrual cycle were selected for dosing with neutron-activated talc for 30 consecutive workdays. Menstrual cycles were determined by inspection of the catch pans under the cages for menstrual blood. The remaining six monkeys served as untreated controls. The monkeys were 4- to 12-yr-old exbreeders (multiparae), ranging in weight from 2.4 to 4.35 kg.

After sedation (25 mg ketamine hydrochloride, intramuscular) each of the six dosed monkeys was placed on her back and restrained by taping hands and tail to a plywood restraining cross. The pelvis was elevated at an angle of about 20 to 25°. The legs were held with the knees bent close to the chest, using a Velcro strap as a restraining mechanism. A nasal speculum was inserted into the vagina and opened to expose the cervix. Each of the six animals received approximately 125 mg neutron-activated talc, suspended in 0.3 ml physiological saline solution containing 1% CMC. The suspension was deposited in the posterior fornix of the vagina, using a 1.0-cm$^3$ Tuberculin syringe with a stainless-steel animal feeding needle (CVD 18 ga. × 0.5 in; Popper & Sons, Inc., New Hyde Park, NY). Once a week, 10 units of oxytocin were injected intramuscularly at the same time as the talc deposition. Following dosing, the animals were maintained in the restrained position for approximately 20 min and then returned to their cages.

Two days after the thirtieth talc deposition, the six dosed animals were anaesthetized by intramuscular injection of 100 mg (1 ml) ketamine hydrochloride and weighed. The abdominal area was shaved. To recover talc particles that may have translocated to the peritoneal cavity, peritoneal lavage was performed by injecting approximately 135 ml physiological saline solution into the peritoneal cavity, followed by brief gentle massage to distribute the lavage fluid and wash off any talc particles which might have adhered to the serous membranes of the peritoneal cavity. The peritoneal cavity was then opened by incision and the lavage fluid collected by aspiration with a syringe for γ-ray analysis. The lavage was repeated once through the abdominal incision.

Precautions to avoid contamination and cross-contamination of samples included the use of clean instruments for each sample to be collected and starting with the collection of samples least likely to contain translocated talc, i.e. lavage fluid and ovaries. Both ovaries were collected in one polyethylene vial for γ-ray analysis. Both oviducts were similarly collected and sectioned into three parts of approximately equal length for γ-ray analysis, followed by collection of the body of the uterus. Because deposition of talc in the area of the vaginal fornix might also result in the direct mechanical deposition (rather than physiological translocation) of talc in the uterine cervix, the cervix of the uterus was dissected from the body and analysed together with the vagina. Thus, the following seven samples from each of the animals were analysed: peritoneal lavage fluid (Sample 1); right and left ovaries, combined (Sample 2); three sections of right and left oviducts (right and left corresponding sections combined in Samples 3a, 3b and 3c; Sample 3a contained the two oviduct sections adjacent to the ovaries, Sample 3c those adjacent to the uterus); body of the uterus (Sample 4); and vagina with cervix (Sample 5). Treated and control animals were then killed by iv injection of a barbiturate-based solution.

Tissue samples were collected for γ-ray analysis in labelled, acid-cleaned polyethylene vials, dried and heat-sealed before analysis, using an infra-red heat lamp. Peritoneal lavage samples were evaporated to approximately 2.5 ml of liquid. Bulk talc standards and liquid standards of iron, cobalt, chromium and scandium in geometrical arrangements similar to those of the samples were analysed on each detector system used for sample analysis. Counting times ranged from 1000 to 2000 min, depending on the activity in the samples.

The samples were counted on two different detector systems. The first was a unique high-resolution, low-background intrinsic germanium (IG), or a lithium-drifted germanium [Ge(Li)] detector with either a NaI(Tl) or plastic phosphor anti-coincidence shield. This system separates the γ-rays emitted into one of two spectral regions. Those γ-rays detected

simultaneously in both the IG [or Ge(Li)] detector and the NaI(Tl)—or plastic phosphor—anti-coincidence shield are stored in the second spectral region. The $\gamma$-rays detected only by the IG [or Ge(Li)] detector are stored in the first spectral region. The great advantage of this system is the reduction of the Compton background by one order of magnitude in the non-coincident portion of the spectrum, resulting in greater sensitivity. The second system was a low-level, ultra-low background NaI(Tl) $\gamma$-$\gamma$ coincidence multi-parameter detector system. This combination provides unmatched sensitivities for the detection of very low-level radioisotope activities. The anti-coincidence system preferentially detects non-coincident $\gamma$-rays ($^{59}$Fe, $^{51}$Cr) whereas the multi-parameter system is designed to preferentially detect coincident $\gamma$-rays ($^{46}$Sc; $^{60}$Co). Because of the time elapsed from the irradiation of the talc and the decay of the relatively short-lived radioisotopes $^{51}$Cr and $^{59}$Fe ($t_{1/2} = 27.7$ and 44.5 days, respectively), two anti-coincidence detector systems were used to expedite $\gamma$-ray analysis. The signals were fed through the appropriate electronics to a 4096-channel analyser that was interfaced to a PDP 11/44 computer for data storage and subsequent data analysis. As mentioned, the second detector system was a NaI(Tl) $\gamma$-$\gamma$ coincidence multi-parameter system. Again, two nearly identical (multi-parameter) systems were used to expedite the counting. The detectors were interfaced via the appropriate electronics to a 4096-channel multi-parameter analyser and the data transferred to magnetic tape. This tape was read into the PDP 11/44 computer for subsequent data analysis. The counting systems were standardized using aliquots of known concentrations of NBS traceable standards obtained from Amersham Corporation ($^{60}$Co, $^{51}$Cr, and $^{59}$Fe) and New England Nuclear Corporation ($^{46}$Sc).

When the infrared heat lamp was turned off, two ovaries were found on the counter next to their sample vials. These ovaries apparently had "popped" out of their vials during the drying process. Without means to determine which ovary was from what animal, these two ovaries were labelled X1 and X2 and treated as separate samples.

## RESULTS

A $\gamma$-ray spectrum of the irradiated talc is shown in Fig. 1. Various isotopes are identified, but the most suitable isotopes for our purposes were $^{46}$Sc, $^{51}$Cr, $^{59}$Fe and $^{60}$Co. A typical spectrum from the anti-coincidence detector system for the sample from monkey No. 81-086-5, which had the most direct contact with the deposited talc, namely the vagina with cervix (Sample 5), is shown in Fig. 2. Measurable quantities of $^{46}$Sc, $^{51}$Cr, $^{59}$Fe, and $^{60}$Co were found in this sample. The peaks of $^{59}$Fe and $^{51}$Cr are readily apparent in the non-coincidence portion of the spectrum. A typical spectrum from the body of the uterus (Sample 4) of monkey No. 81-081 is shown in Fig. 3. The $\gamma$-rays, associated with the previously mentioned radioisotopes characteristic of talc, are not present. Instead, its spectrum closely resembles the background spectrum shown in Fig. 4, which is from the vagina and cervix (Sample 5) of control monkey no. 79-280 in which only background radioisotopes were present.

Radioisotope data for $^{46}$Sc and $^{60}$Co from the multi-parameter system, and $^{59}$Fe and $^{51}$Cr data from the anti-coincident systems, are combined in Tables 3 and 4 for the experimental and control samples, respectively. Where applicable, a 'less-than' value is reported. This value is based on one standard deviation of the background observed in the collected spectrum.

Once the most representative values for the samples had been determined, the results were converted from dpm to $\mu$g of talc where applicable. This conversion was based on the radionuclide concentration in the talc, namely: $0.316 \pm 0.7\%$ for $^{46}$Sc, $2.29 \pm 0.9\%$ for $^{51}$Cr, $0.617 \pm 0.7\%$ for $^{59}$Fe, and $0.297 \pm 0.9\%$ dpm per $\mu$g of talc for $^{60}$Co. The conversion of the observed activity (dpm per sample) to $\mu$g of talc per sample was made as follows:

$$M_{talc} = (A_{net})_{element}/(A_{talc})_{element}$$

where $M_{talc}$ = mass of talc in $\mu$g talc/sample, $A_{net}$ = net decay-corrected activity in dpm/sample, and $A_{talc}$ = decay-corrected activity of talc in dpm/$\mu$g of talc.

The quantities of talc per sample have been reported, where applicable, in Table 3. As expected, measurable quantities of talc were observed in the vagina + cervix sample (Sample 5) from each dosed monkey. Their quantity was estimated using the values for $^{46}$Sc and $^{60}$Co reported for each sample. The observed quantities of talc for these samples were 77,000, 117,000, 63,000, 470, 18 and 6 $\mu$g of talc. These wide variations were most likely due to different phases of the animals' menstrual cycles at the time of death, with menstrual flow cleansing the vagina of much of the deposited talc. No measurable levels of the activated talc were present in any of the other samples.

## DISCUSSION

The oviducts provide a passage from the ovaries and the peritoneal cavity to the uterus and the vagina. This pathway can be travelled by cells in either direction as demonstrated by ova and spermatozoa. Gases and liquids such as radio-opaque contrast material and dyes can also be passed by appropriate manipulation through the cervix into the peritoneal cavity. It is less clear whether or not inanimate particles such as carbon black or talc can translocate of their own accord from the vagina to the oviducts and beyond.

As already mentioned, in two of three cases Egli & Newton (1961) found carbon particles in the liquid with which they flushed the oviducts of three patients half an hour after carbon black deposition in the vagina. Theirs was a non-quantitative study that did not include the examination of liquid or filter blanks as negative controls. In a similar experiment with cynomolgus monkeys, we observed approximately equal quantities of carbon black particles in the flushing liquid as well as in our liquid blanks. In light of our previous findings (Wehner *et al.* 1985), it is possible that Egli and Newton might have observed false positives due to sample contamination.

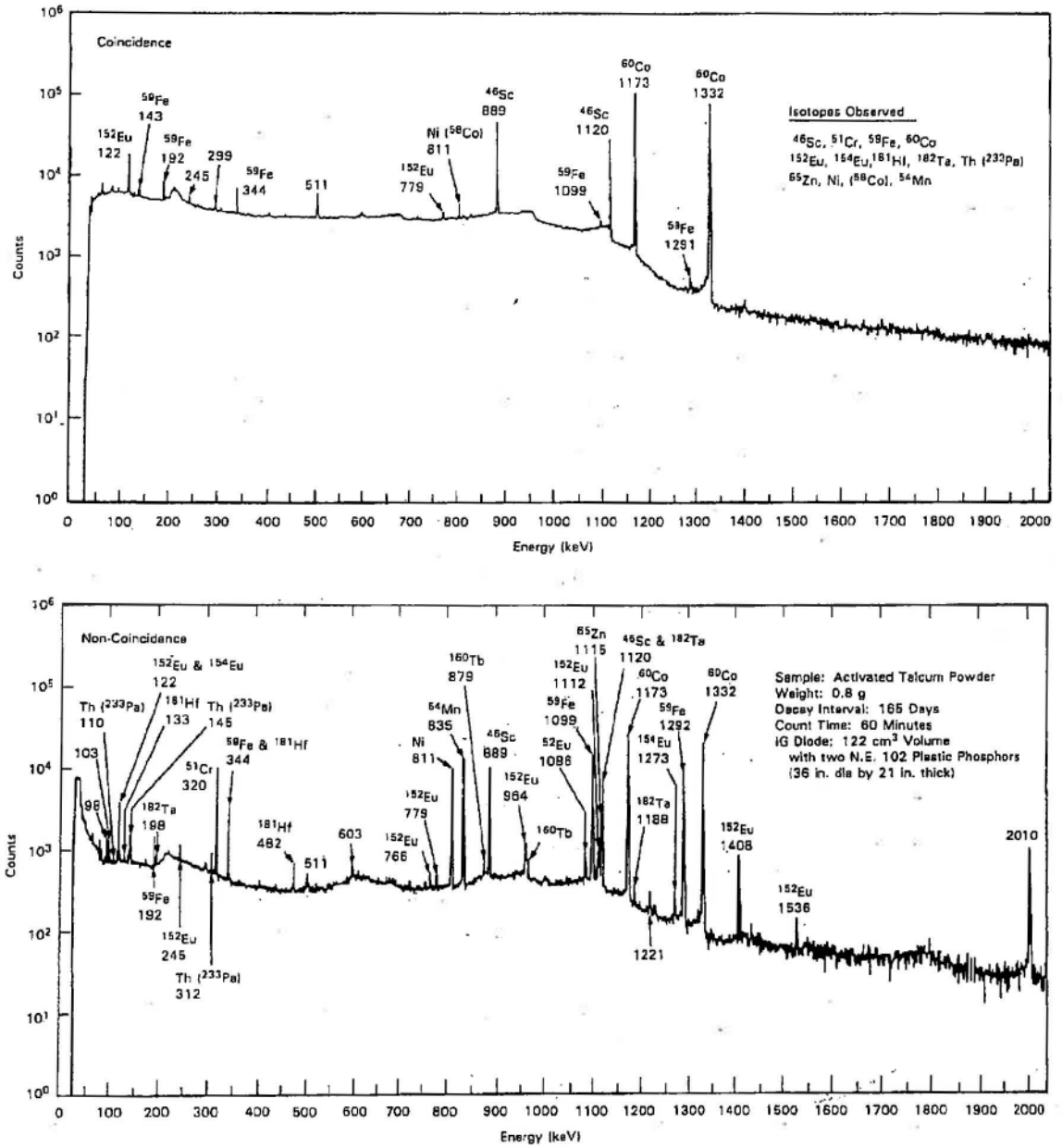


Fig. 1. Gamma-ray spectrum of neutron-activated talc.

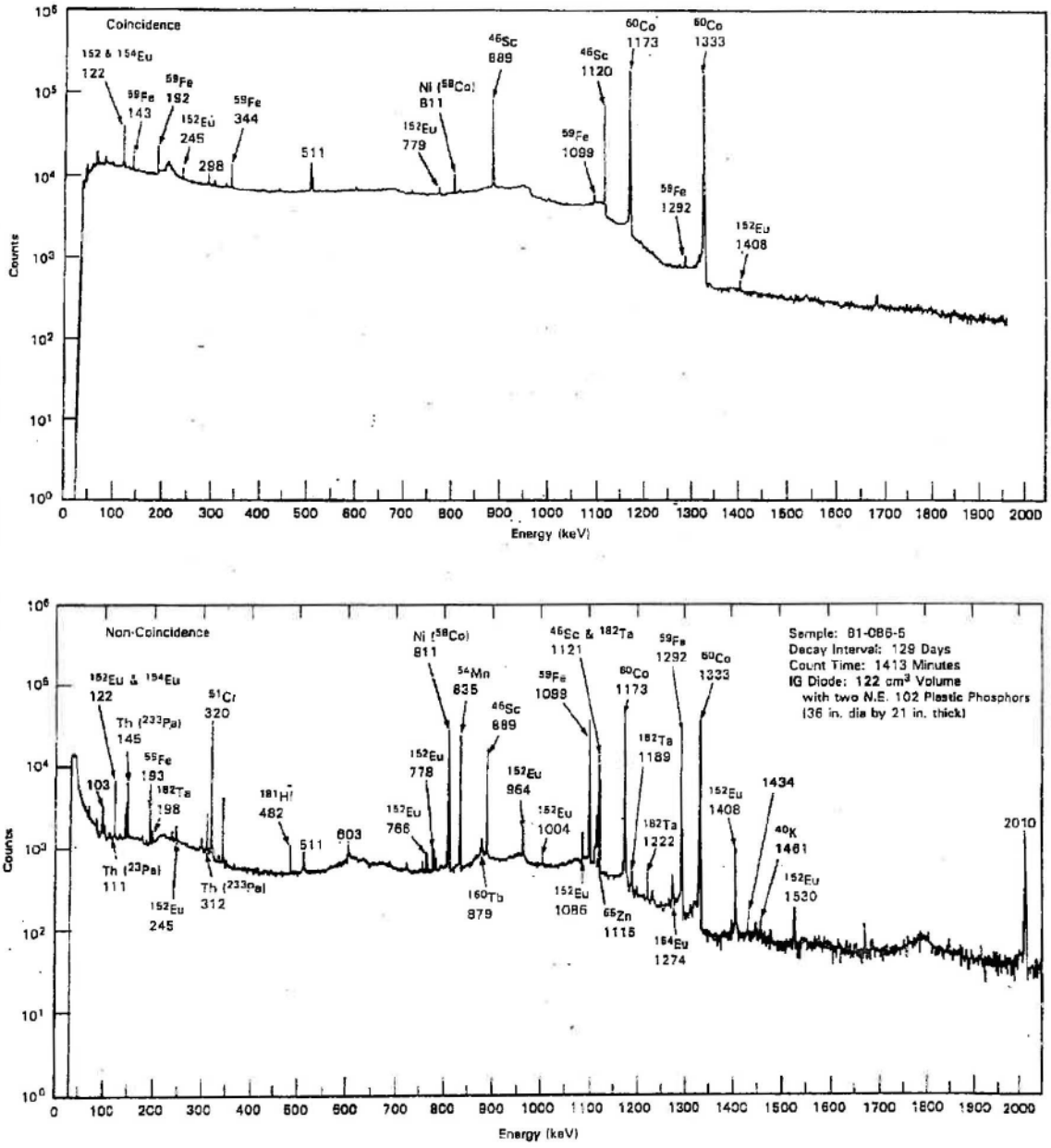


Fig. 2. Gamma-ray spectrum of Sample 5 (vagina + cervix) from monkey 81–086, which had received 30 applications of neutron-activated talc.

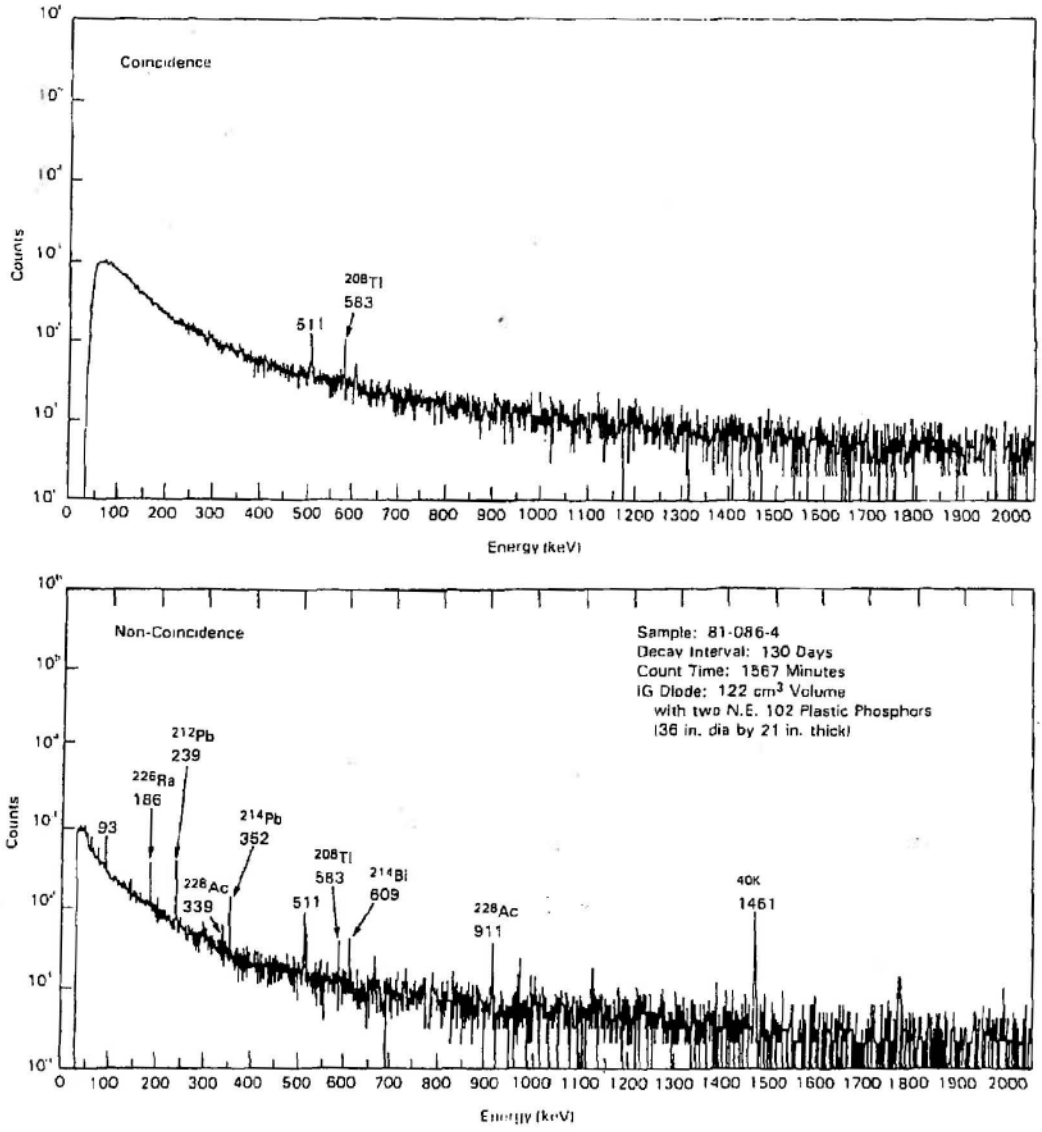


Fig. 3. Gamma-ray spectrum of Sample 4 (body of uterus) from monkey 81–081, which had received 30 applications of neutron-activated talc.

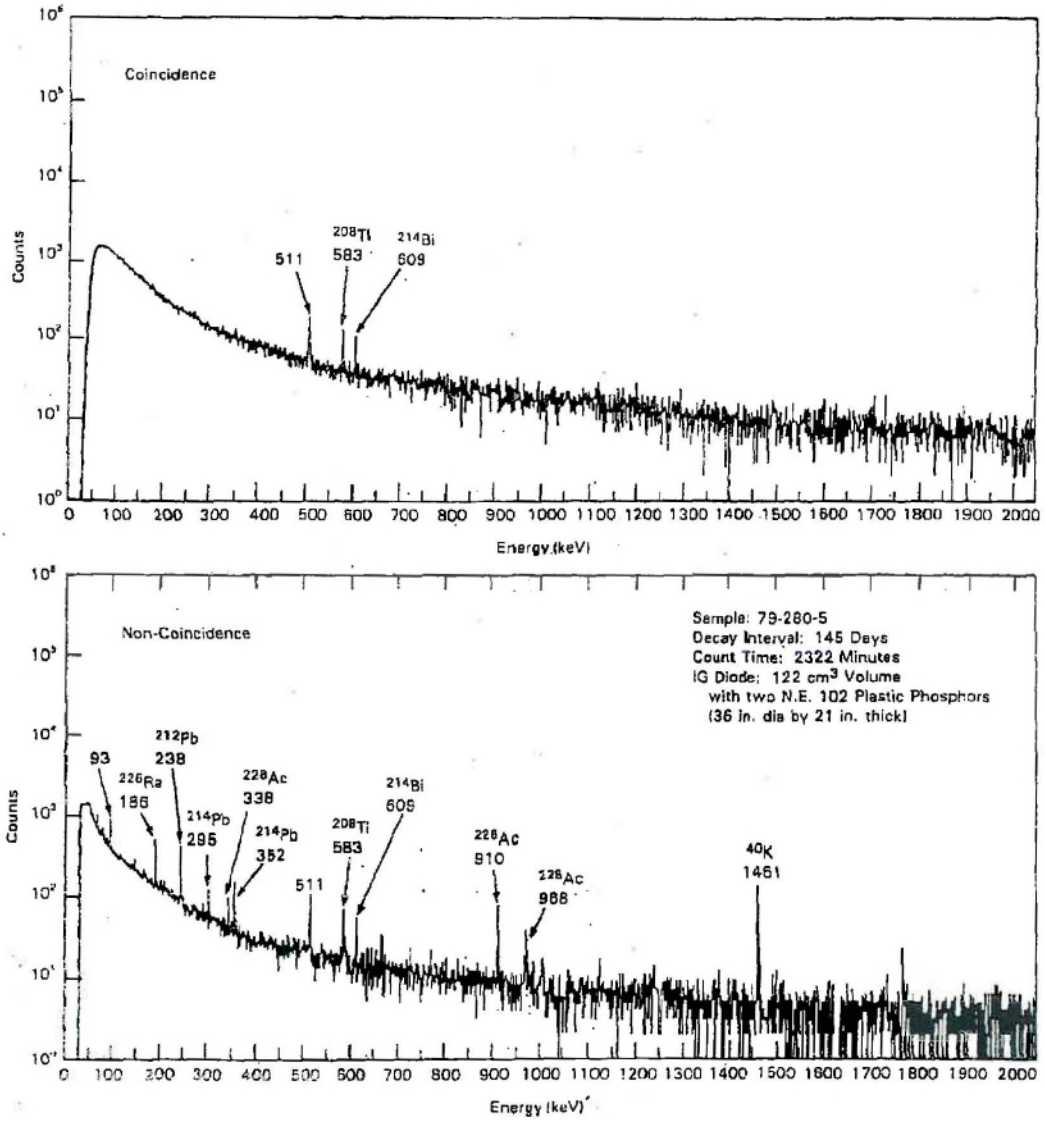


Fig. 4. Gamma-ray spectrum of Sample 5 (vagina + cervix) from control monkey 79–280.

Table 3. Best activity values observed in tissue samples and peritoneal lavage fluid from monkeys treated with neutron-activated talc by vaginal deposition

| Monkey and sample numbers* | Activity (mean dpm/sample ± SD) | | | | |
|---|---|---|---|---|---|
| | Scandium | Chromium | Iron | Cobalt | Average |
| **79–252** | | | | | |
| 1 | <0.26 | <81 | <4.5 | 0.40 ± 0.13 | |
| 2 | 1.1 ± 0.4 | <41 | <3.1 | 0.52 ± 0.15 | |
| 3a | <0.24 | <60 | <3.7 | <0.11 | |
| 3b | <0.26 | <41 | <2.7 | <0.11 | |
| 3c | <0.22 | <44 | <2.4 | <0.11 | |
| 4 | <0.20 | <70 | <4.8 | <0.11 | |
| 5 | 22000 ± 200 | 342600 ± 500 | 66900 ± 1300 | 25010 ± 15 | |
| *μg of talc* | 69600 ± 600 | 124500 ± 500 | 128700 ± 800 | 84210 ± 50 | 77000 ± 10000 |
| **79–256** | | | | | |
| 1 | 1.0 ± 0.3 | <68 | <3.7 | <0.13 | |
| 2 | <0.30 | <56 | <3.9 | <0.12 | |
| 3a | <0.28 | <63 | <3.7 | <0.13 | |
| 3b | <0.23 | <42 | <2.9 | <0.11 | |
| 3c | <0.27 | <30 | <2.2 | <0.11 | |
| 4 | 0.61 ± 0.25 | <60 | <4.5 | <0.11 | |
| 5 | 34370 ± 350 | 382700 ± 1800 | 104300 ± 2000 | 37330 ± 50 | |
| *μg of talc* | 108800 ± 1100 | 189100 ± 600 | 188000 ± 1300 | 125700 ± 200 | 120000 ± 12000 |
| **81–086** | | | | | |
| 1 | <0.25 | <101 | <5.5 | <0.11 | |
| 2† | <0.29 | <68 | <3.7 | <0.11 | |
| 3a | <0.33 | <85 | <4.8 | <0.13 | |
| 3b | <0.27 | <45 | <2.8 | <0.13 | |
| 3c | <0.26 | <37 | <2.5 | <0.12 | |
| 4 | <0.18 | <70 | <3.7 | <0.10 | |
| 5 | 18300 ± 200 | 241300 ± 500 | 47500 ± 1100 | 20500 ± 20 | |
| *μg of talc* | 57900 ± 600 | 101700 ± 400 | 99100 ± 400 | 69020 ± 70 | 63000 ± 8000 |
| **81–092** | | | | | |
| 1 | <0.22 | <85 | <5.7 | <0.11 | |
| 2 | <0.29 | <52 | <3.2 | <0.11 | |
| 3a | <0.31 | <74 | <3.9 | <0.12 | |
| 3b | <0.29 | <49 | <3.3 | <0.12 | |
| 3c | <0.25 | <35 | <2.6 | <0.10 | |
| 4 | <0.20 | <60 | <4.0 | <0.11 | |
| 5 | 138 ± 2 | 1090 ± 200 | 342 ± 8 | 150 ± 1 | |
| *μg of talc* | 437 ± 6 | 560 ± 40 | 760 ± 30 | 505 ± 5 | 470 ± 50 |
| **81–102** | | | | | |
| 1 | <0.26 | <93 | <6.0 | <0.12 | |
| 2 | <0.24 | <57 | <3.0 | <0.09 | |
| 3a | <0.27 | <85 | <4.1 | <0.10 | |
| 3b | <0.21 | <47 | <3.0 | <0.11 | |
| 3c | <0.23 | <35 | <2.6 | <0.11 | |
| 4 | <0.19 | <55 | <4.4 | <0.09 | |
| 5 | 8.6 ± 0.5 | <60 | 13 ± 5 | 2.6 ± 0.2 | |
| *μg of talc* | 27 ± 2 | <26 | 21 ± 8 | 8.7 ± 0.7 | 18 ± 13 |
| **81–166** | | | | | |
| 1 | <0.22 | <86 | <5.5 | <0.10 | |
| 2† | <0.33 | <83 | <4.5 | <0.12 | |
| 3a | <0.32 | <64 | <3.5 | <0.11 | |
| 3b | <0.22 | <66 | <3.8 | <0.11 | |
| 3c | <0.23 | <41 | <3.0 | <0.11 | |
| 4 | <0.23 | <70 | <4.5 | <0.11 | |
| 5 | 2.1 ± 0.3 | <60 | <4.7 | 1.4 ± 0.1 | |
| *μg of talc* | 6.6 ± 1.0 | <26 | <7.6 | 4.7 ± 0.3 | 5.7 ± 1.3 |
| X1 | <0.31 | <45 | <3.1 | 0.27 ± 0.12 | |
| X2 | <0.31 | <46 | <2.9 | <0.10 | |

†Sample numbers: (1) peritoneal lavage fluid, (2) right and left ovaries combined, (3a, 3b and 3c) three sections of right and left oviducts, (4) body of the uterus, and (5) vagina with cervix.
†One of the two ovaries "popped" out of the vial during the drying process. The activities of the popped out ovaries are listed as X1 and X2.

DeBoer (1972) deposited 0.2 ml of a colloidal carbon black suspension in the uterine cavity, the cervical canal or the vagina of well over 100 patients prior to abdominal surgery. Subsequent macroscopic examination of the oviducts showed rapid translocation of the carbon black deposited in the uterus to the oviducts and beyond in the majority of the cases. Some of the carbon black deposited in the cervical canal also translocated, but to a lesser extent. However, "from the vagina to the uterus passage of the marker was observed only twice in thirty-seven investigations." DeBoer pointed out that his patients were placed in the Trendelenberg position after the abdomen had been opened and that "in this position, especially under anaesthesia, there is a negative intra-abdominal pressure which may be sufficient to draw

Table 4. Best activity values observed in tissue samples and peritoneal lavage fluid from control monkeys

| Monkey and sample numbers* | Activity (dpm/sample) Scandium | Chromium | Iron | Cobalt |
|---|---|---|---|---|
| **77–403** | | | | |
| 1 | <0.34 | <150 | <6.1 | <0.13 |
| 2 | <0.35 | <87 | <4.3 | <0.11 |
| 3a | 3910 ± 20 | <86 | <4.4 | <0.12 |
| 3b | <0.33 | <64 | <3.5 | <0.10 |
| 3c | <0.27 | <79 | <3.8 | <0.08 |
| 4 | <0.25 | <100 | <5.2 | <0.09 |
| 5 | <0.25 | <110 | <5.6 | <0.11 |
| **77–091** | | | | |
| 1 | <0.28 | <140 | <6.5 | <0.10 |
| 2 | <0.40 | <160 | <6.3 | 1.3 ± 0.2 |
| 3a | <0.35 | <130 | <5.6 | <0.11 |
| 3b | <0.40 | <97 | <4.2 | <0.13 |
| 3c | <0.54 | <180 | <7.4 | <0.18 |
| 4 | <0.27 | <100 | <5.7 | <0.10 |
| 5 | <0.30 | <100 | <5.1 | <0.13 |
| **79–280** | | | | |
| 1 | <0.26 | <140 | <6.9 | <0.09 |
| 2 | <0.45 | <150 | <6.1 | <0.14 |
| 3a | <0.35 | <180 | <7.2 | <0.11 |
| 3b | <0.43 | <130 | <5.0 | <0.14 |
| 3c | <0.34 | <170 | <6.0 | <0.11 |
| 4 | <0.28 | <140 | <9.1 | <0.10 |
| 5 | <0.22 | <87 | <4.9 | <0.11 |
| **80–053** | | | | |
| 1 | <0.26 | <160 | <7.7 | <0.09 |
| 2 | <0.25 | <180 | <8.0 | <0.07 |
| 3a | <0.30 | <190 | <8.2 | <0.08 |
| 3b | <0.43 | <180 | <7.2 | <0.12 |
| 3c | <0.43 | <160 | <7.2 | <0.12 |
| 4 | <0.25 | <130 | <6.0 | <0.09 |
| 5 | <0.22 | <120 | <6.7 | <0.10 |
| **80–087** | | | | |
| 1 | <0.31 | <140 | <6.8 | 0.71 ± 0.13 |
| 2 | <0.40 | <110 | <4.8 | <0.22 |
| 3a | <0.36 | <150 | <6.1 | <0.11 |
| 3b | <0.40 | <160 | <6.7 | <0.11 |
| 3c | <0.40 | <140 | <6.6 | <0.11 |
| 4 | <0.21 | <130 | <6.1 | <0.08 |
| 5 | <0.19 | <140 | <6.3 | <0.08 |
| **81–164** | | | | |
| 1 | <0.38 | <160 | <6.7 | <0.13 |
| 2 | <0.44 | <150 | <6.7 | <0.11 |
| 3a | <0.40 | <140 | <7.1 | <0.11 |
| 3b | <0.44 | <120 | <5.3 | <0.12 |
| 3c | <0.45 | <110 | <4.6 | <0.12 |
| 4 | <0.29 | <130 | <7.9 | <0.12 |
| 5 | <0.29 | <110 | <5.2 | <0.11 |

*Sample numbers: see Table 3 footnote.

up material from the vagina into the uterus, particularly through a relaxed cervix." He further pointed out that one of these two positive patients was a multipara (six children) with a lacerated cervix. DeBoer's results tend to support our findings by indicating that the cervical canal represents a formidable barrier to the translocation of insoluble inanimate particles from the vagina to the uterus.

Hassler *et al.* (1974) observed transcervical migration of $^{125}$I- or $^{55}$Sr-labelled microcapsules in rabbits and in some but not all stumptail monkeys and baboons when the sedated primates were maintained in their supine positions for 1 or 6 hr following dose administration (Gardner *et al.* 1980). When migration did occur, it varied greatly from animal to animal and was on the order of 1% or less during the first 24-hr period following dosing. The difference between our results and those reported by Gardner *et al.* (1980) may be due to differences in experimental procedures; Gardner *et al.* administered considerably higher doses per application (~1 g), used markedly different materials and a longer sedation time, and maintained the primates much longer in a supine position after dosing.

Venter & Iterralde (1979) placed $^{99m}$Tc-labelled human albumin microspheres (HAM) in the vaginas of patients, followed by surgical removal of uterus, oviducts and ovaries. These tissues/organs were then analysed for $^{99m}$Tc, using a scintillation detector. In 9 of 14 cases, radioactivity levels were detected in the oviducts and ovaries; the remaining five cases were negative. All negative cases occurred in patients with proven oviduct changes due to previous infection. While Venter & Iterralde (1979) provide strong suggestive evidence for the translocation of microspheres from the vagina to the oviducts and ovaries, their case is not necesarily conclusive. This statement is based on the observation that the activity from a single radionuclide label measured in organs/tissues does not necessarily prove the presence of particles because radionuclides can leach from the particles (Subramanian, Rhodes, Cooper & Sodd, 1975; Wehner & Wilkerson, 1981; Wehner, Wilkerson, Cannon *et al.* 1977; Wehner, Wilkerson, Mahaffey & Milliman, 1980; Wehner, Wilkerson & Stevens, 1984) as specifically demonstrated for $^{99m}$Tc-labelled HAM (Bolles, Kubiatowicz, Evans *et al.* 1971). Misleading conclusions due to the dissociation of radionuclide labels from test materials can be avoided by monitoring for more than one radionuclide. Comparing the ratios of several radionuclides-to-test-material in the bulk material to these ratios in the material deposited in any given tissue will reveal leaching because each radionuclide dissociates at a different rate from a given material (Wehner & Wilkerson, 1981; Wehner *et al.* 1977, 1980 & 1984).

Henderson *et al.* (1971) found talc particles in 10 of 13 ovarian tumours in humans, using an extraction-replication technique (Henderson, 1969). Cramer, Welsh, Scully & Wojciechowski (1982) observed a statistically significant ($P < 0.003$) relationship between epithelial ovarian cancer and talc used for dusting the perineum or sanitary napkins in 215 women. Both of these two clinical studies imply translocation of talc to the ovaries. However, Cramer *et al.* (1982) found no relationship between ovarian cancer and talc exposure from dusting condoms or diaphragms, even though talc, in the latter applications, is deposited close to the cervical os. Hartge, Hoover, Lesher & McGowan (1983) made a similar observation from their epidemiological study. Their data indicated that the use of talc on a diaphragm did not appear to elevate risk and that there was no overall association between talc use and risk of ovarian cancer. Phillips, Young, Hardy & Gangolli (1978) found no translocation of $^{3}$H-labelled talc from the vagina to ovaries in the rabbit.

None of these studies conclusively answers the question of whether or not talc, deposited in the vagina of the human female, translocates to the oviducts and beyond without purposeful manipulation. Our study, using state-of-the-art techniques in the most suitable animal model available, failed to

provide any evidence for such translocation of measurable quantities ($> \sim 0.5\,\mu g$, depending on the radionuclide, detector system and counting time) of talc.

It would, indeed, be difficult to explain such a translocation of "insoluble" inanimate particles. They lack the locomotion of spermatozoa and are unable to respond to chemotactic or physiological stimuli. It is, therefore, reasonable to assume that the behaviour of such particles is largely governed by the laws of physics. These laws would not permit particles to migrate "upstream" against the direction of the beat of the oviduct's ciliary epithelium, even if the particles had managed to somehow breach the cervical barrier and diffuse across the uterine cavity.

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# Exhibit F

REGULATORY TOXICOLOGY AND PHARMACOLOGY 21, 242–243 (1995)

# The Lack of an Ovarian Effect of Lifetime Talc Exposure in F344/N Rats and B6C3F1 Mice[1]

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**TABLE 1**
**Incidence of Ovarian Lesions in Female Rats**

| Diagnoses | 0 mg/m³ | 6 mg/m³ | 18 mg/m³ |
|---|---|---|---|
| Ovarian cyst | 5 | 0 | 1 |
| Granulosa cell tumor, malignant | 1 | 0 | 0 |
| Granulosa cell tumor, benign | 0 | 2 | 0 |
| Granulosa-theca tumor, benign | 0 | 1 | 0 |
| Granulosa-theca tumor, malignant | 0 | 0 | 1 |

**TABLE 2**
**Incidence of Ovarian Lesions in Female Mice**

| Diagnoses | 0 mg/m³ | 6 mg/m³ | 18 mg/m³ |
|---|---|---|---|
| Ovarian cyst | 6 | 11 | 10 |
| Abscess | 4 | 10 | 7 |
| Thrombosis | 1 | 2 | 0 |
| Adenoma | 1 | 1 | 0 |
| Cystadenoma | 0 | 1 | 0 |
| Luteoma | 2 | 0 | 0 |

There has been some concern reported about the perineal exposure to talc and the occurrence of ovarian cancer in women (Cramer *et al.*, 1981; Henderson *et al.*, 1971), although other studies have failed to find such an association (Whittemore *et al.*, 1988). Talc particles have also been reported to be present in the ovaries of women regardless of history of talc exposure (Heller *et al.*, 1994). An NTP study of lifetime whole body exposure talc exposure (NTP, 1993) offered the opportunity to determine whether rodents would have ovarian talc particles after inhalation, oral, and dermal exposure for more than 2 years.

Male and female Fisher 344/N rats and B6C3F1 mice were exposed to aerosol concentrations of 0, 6, or 18 mg/m³ of talc for lifetime (rats) or 2 years (mice). There were no exposure-related lesions in the ovaries of rats (Table 1) or mice (Table 2); however, because of the concern of potential effects of talc on the ovary, additional studies were performed.

Ten female rats were selected randomly from the control and exposure groups of 6 and 18 mg/m³, and the histological slides containing the lungs and ovaries were examined under polarized light for the presence of anisotropic material consistent with talc particles. The lungs from the controls were negative for anisotropic materials but talc particles were easily identified from the lungs of the exposed animals. The particles were present in the alveolar macrophages and in areas associated with chronic inflammation in the lungs. There was no material consistent with talc found in the ovaries or ovarian bursa from any rats from any group.

This would suggest that extensive lifetime exposure to talc does not result in the deposition of talc in the ovary. Since the animals were exposed for 6 hr per day with talc covering the fur and the cage bars, there was ample opportunity for perineal as well as oral and respiratory exposure. In the extrapolation of these data one should consider limitations relative to the marked anatomical and physiological differences betwsen rodents and humans.

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